JOURNAL OF THE KENTUCKY ACADEMY OF SCIENCE

Official Publication of the Academy

KAS

Volume 72

Number 2

Fall 2011

The Kentucky Academy of Science

Founded 8 May 1914

Governing Board 2011

⊗ This paper meets the requirements of ANSI/NISO Z39.48-1992 (Permanence of Paper).

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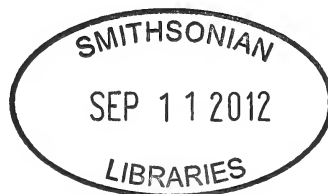Thomas More College

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A Study of a Common Misconception in Appalachian Kentucky Seventh and Eighth Grade Science Students: Free Fall and Inertia

Jessica C. Lair[1] and Jerry D. Cook

Department of Physics and Astronomy, Eastern Kentucky University, Richmond, Kentucky 40475

ABSTRACT

Since the inception in 1990 of the Kentucky Education Reform Act, the Commonwealth of Kentucky has taken steps to improve middle-school student education, particularly in science. In this study, we evaluated the effects that these changes on middle school student performance, particular with respect to misconceptions about free fall and inertia. We collected data from approximately 750 seventh- and eighth-grade students from three middle schools in south-central Kentucky. We then surveyed the students, using a series of questions related to force and motion, after they had completed coursework at the relevant grade level. Overall, we found that students had a poor understanding of these topics. Seventh graders tended to perform better than their eighth grade counterparts, and male students tended to perform better than female students. We also conducted video interviews with a select number of students to evaluate their understanding of free fall and inertia. The video interviews showed the depth of misconceptions. We found that those students retained these misconceptions even after having completed the appropriate coursework.
KEY WORDS: Science education, misconceptions, free-fall, middle-school education, Kentucky

The persistence of misconceptions in science, particularly physics, has been the topic of many studies (Confrey 1990; Pfundt and Duit 1993; Driver, Squires, Rushworth and Wood-Robinson 1994; Ram, Nersessian and Keil 1997; Reiner, Slotta, Chi and Resnick 2000). Numerous researchers have studied how children learn science (Duschl, Schweingruber and Shouse 2007). Several studies have evaluated how students at different education levels overcome scientific misconceptions. In a study involving university engineering students, McCloskey (1983) found that more than 50 percent of students retained their misconceptions about force, even after they had completed a semester of university physics. Other studies (Anderson and Smith 1987; Chi 2005) showed that these misconceptions are stable, robust, and resistant to instruction.

In this study, we report on what we consider to be the most persistent misconception in physics, i.e., heavy objects fall faster than light objects. We collected data from three middle schools in the Appalachian region of Kentucky that we considered to be representative of most middle schools based on demographic data (see Table 1). We combined data collected from written forms and oral interviews to evaluate seventh and eighth grade students who have completed science for the academic

term. Our goal was to determine the extent of perceived misconceptions and to try to determine why these misconceptions were so hard to overcome. We found that there are powerful reasons why students tend to retain misconceptions about free-fall.

In 1990, the Kentucky Supreme Court ruled that the current funding system in Kentucky schools was unconstitutional due to the inequity of resources that were available to different school districts. The court found that there were significant differences between wealthy counties, such as those in central Kentucky, and poor counties, such as those in rural school systems located in southern and eastern parts of the state. The Kentucky Educational Reform Act (KERA 1990) was designed to rectify this funding disparity and to place all school districts on an equal funding basis. One part of the Act was intended to document improvements in learning among educational systems in the Commonwealth of Kentucky.

The Act included provisions for improving the Science, Technology, Engineering, and Math (STEM) disciplines. The Act was initiated in concert with the nationwide trend toward developing and implementing new ways of teaching science in the middle school grades, including inquiry learning, small group activities, and peer instruction (e.g., Krajcik et al. 1998; Krajcik et al. 2000; Linn et al. 2003). Educators have instituted a new

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[1] To whom correspondence should be addressed. E-mail: jessica.lair@eku.edu

Table 1.   Demographics of schools participating in study.

School	Enrollment	Participants	% free lunch
One	447	236	57
Two	618	418	74
Three	785	92	48

method for teaching elementary and middle school grades based on the Kentucky Core Content for Assessment. Since the enactment of the KERA initiative, the Commonwealth of Kentucky has undertaken several steps to address the inadequacies in K-12 science classes.

The Commonwealth Accountability Testing System or CATS exam, which consists of a series of examinations given at periodic intervals throughout student careers, was used as the primary indicator for determining success or failure. In science, specifically, proficiency examinations were given at the fourth, seventh, and eleventh grade levels. We propose that this study can be used as an indicator for determining how effective the recent changes educational methodologies have been in eliminating common misconceptions in science and to gauge how well seventh and eighth grade students retain new information.

We also attempted to answer the question, "Are students entering high school at the ninth grade level prepared to do well in introductory science courses?" We used a simple set of questions to measure their preparation. We contend that our findings should be part of a wider study conducted by other institutions in different sections of the country. We invite work from other researchers, and we encourage them to compare their results to our findings.

In this study, we collected written responses from seventh and eighth grade students along with oral interviews from selected students. The purpose of these interviews was to evaluate existing models and use this material to make suggestions on ways to improve science teaching at the middle school level.

METHODS AND MATERIALS

We tested approximately 750 students in three different Appalachian middle schools at the seventh and eighth grade levels to determine the prevalence of misconceptions regarding falling objects, gravitational acceleration, and inertia. Numerous studies have addressed these misconceptions throughout a student's elementary and middle school tenure. In our study, we ask the question, "How often do students give a correct response when posed with questions related to objects falling to earth?"

The answers to this question may provide a useful indicator of students' understanding, or lack of understanding, of force and motion. We chose this question, in part, based on informal surveys from college-level students who were taking an inquiry-based physics class for elementary and middle grade pre-service teachers. The surveys showed that 50% of the students in some classes believed that heavier objects fell faster than lighter objects. Even among students who stated that all objects fall at the same rate, there was no self-consistent model that distinguished "regular fall," in which air resistance must be considered, from "free fall." When these same students were asked why they thought all objects fall at the same rate, they stated that the pull of gravity was the same on all objects. In these cases, students had pushed their misconceptions about free fall to another level. We decided to test student misconceptions about "free fall" and "regular fall" based on this anecdotal evidence obtained from post secondary students.

ADMINISTRATION AND ACCESS

In this study, we had access to six different middle schools in the vicinity of Madison County, Kentucky, under the National Science Foundation-funded project, "Enhancing Inquiry-Based Science and Math in Appalachian Middle Schools (ISMAM)." ISMAM was a collaborative project between Eastern Kentucky University and six middle schools in Appalachian Kentucky (southeastern Kentucky), whose goal was to improve teaching of STEM disciplines in middle schools, specifically inquiry-based instruction (Otieno and Wilder 2010).

Under this program, we were given varied degrees of access to seventh- and eighth-grade students. In this study, we present data from three of these six schools. The schools selected were partner schools in the ISMAM grant and were considered representative of middle

schools and were based on demographic data (see Table 1). We collected data from three of the six schools due to limited resources, access, and time constraints.

We were prevented from identifying the schools under confidentiality agreements; however, we described our findings in Table 1 using brief demographic data. The tables include data on total school enrollment, number of study participants, and percentage of students who received free lunches. Graduate teachings assistants or upper-level undergraduate majors in one of the STEM departments at Eastern Kentucky University administered the pre and post exams and surveys. The proctors were responsible for administering the survey exams and for maintaining student confidentially of written exams and scores. The exams covered the period from 2006 to 2008.

DATA COLLECTION

Seventh and eighth grade students were administered the written examination, which consisted of 12 questions. The last two exam questions were used to evaluate how seriously perceived the exam, i.e., did students take the exam seriously. The administrators, at their discretion, could choose to eliminate any exams that were turned in by unmotivated students. These indicators could be used, at a later date, to validate data on the bottom 25% of students. They also could be used to separate students who did not understand the science from those who did not give the exam a serious effort.

Proctors administered the exams during the latter part of the school year, after students had completed their science requirements related to force and motion. The eighth grade students were finished with middle school science and were preparing to enter high school the following year. The State science proficiency exam was given in the seventh grade; therefore, there were no accountability requirements for middle school science after the seventh grade. We compared seventh and eighth grades scores as an indicator of seventh- and eighth-grade student understanding of science. We contend that these scores can also be used as one indicator of scientific preparedness for students who are preparing to enter high school the following academic year.

INTERVIEWS

We recorded interviews with 18 students from School One then transcribed the interviews for further analysis. We chose School One because of its proximity to us and because the local school administrators ensured us that the parents of the participating students granted us permission to video tape the interviews. The interview questions were designed to examine misconceptions about free fall and inertia and help us understand the meaning of the written portion of the study.

DATA AND ANALYSIS

We used Parscore software from Scantron to score the exams and develop the associated statistics. We used the software to group scores into three groups: the top 25%, the middle 50%, and the bottom 25%. We only reported responses from the upper 75% of the respondents to eliminate those students who not motivated to participate in the study. We used scores from all twelve questions only to separate students into the three groups. Although the entire exam was used to group students, we only used the following four questions because they were directly related to the student's understanding of acceleration, free fall, and inertia.

Question 1

Two small objects are dropped from the same height at the same time. Object one is twice as massive as object two, but they are both the same size. Answer questions one and two with this information.

Which is most true?

a) Both objects will hit the ground at the same time; however, object one will be moving faster than object two just before they strike the ground.

b) Both objects will hit the ground at the same time; however, object two will be moving faster than object one just before they strike the ground.

c) Both objects will hit the ground at the same time, moving at the same speed, just before they strike the ground.

d) Object one will hit the ground before object two because object one is heavier.

Question 2

Which object has the greater acceleration during this fall?

a) Object one
b) Object two
c) Neither object, they have the same acceleration.
d) They both fall at a constant speed and neither accelerates.

Question 3

The Voyager spacecraft has left the solar system and is in empty space where there are no forces. It is most likely to:

a) Move in a straight line forever
b) Coast to a stop
c) Move in a circular orbit around the galaxy
d) There is no way to tell what the spacecraft will do.

Question 4

Suppose you take a trip to the moon. Which of the following is true?

a) Your mass remains the same but your weight changes.
b) Both your mass and weight remain the same.
c) Your mass changes but your weight remains the same.
d) Both your mass and weight decrease.

We chose these questions to determine how well students understood the definition of acceleration, inertia and mass/weight and basics of free fall, and. We believe that the questions on mass and weight and inertia, are related because students who answered questions 1 and 2 correctly may have a misconception about mass and inertia that can be examined in the post-survey interviews.

RESULTS

The following discussions make use of Tables 2 through 6. The tables are broken down in various groups. We separated the results by school and grade level. The data are more extensive for School One compared with Schools Two and Three because we had unlimited access to classrooms in School One.

Table 2. Percentage of students correctly answering question 1.

School	Grade	Correct Responses
One	7	61.1
	7*	70.0
	8	24.0
	8*	86.9
Two	7	22.7
	8	27.0
Three	7	48.2
All	7	41.7
	8	37.7

Note. *classes receiving inquiry-based instruction.

Question 1

Table 2 shows the results of Question 1 by grade level (i.e., 7 or 8) and school. The table also includes a summary of the results for the entire group. We designed Question 1 to test the student's ability to predict a real world event and to separate out misunderstanding created by terminology, particularly acceleration. The question tests students' ability to predict time dependence of objects in free fall, taking into consideration that the students understand that all of the objects will be falling at similar speeds from the same location. Of all the students who took the exam, only 34.6% of seventh graders and 41.7% of eighth graders answered the question correctly.

We did not find any patterns that might suggest that eighth grade students had a better understanding of free fall than their seventh grade counterparts. A comparison of each school's scores showed that the percentage of students who answer the question correctly varied from a high of 86.9% in the inquiry-based eighth grade class at School One to a low of 22.7% in seventh grade class

Table 3. Percentage of students correctly answering question 2.

School	Grade	Correct Responses
One	7	39.0
	7*	61.5
	8	29.2
	8*	49.9
Two	7	19.5
	8	20.2
Three	7	31.6
All	7	31.5
	8	26.6

Note. *classes receiving inquiry-based instruction.

Table 4. Percentage of students correctly answering question 3.

School	Grade	Correct Responses
One	7	49.8
	7*	87.7
	8	24.4
	8*	94.7
Two	7	75.8
	8	52.1
Three	7	43.4
All	7	66.1
	8	57.0

Note. *classes receiving inquiry-based instruction.

at School Two. We expected student who missed this question to choose option "d," (see Table 6). As it turned out, more than 60% of the eighth graders in School 2 choose answer "d," whereas less than 10% of the seventh graders in School One choose option "d." Despite attempts to clarify the misconception that heavy objects fall faster than light objects, students continued to adhere to their misconception, which is based on an intuitive model.

The results obtained from School Two showed that three times as many students believe that heavy objects fall faster than light objects. In School Three, a nearly equal number of students chose answers *c* and *d*. School One had overcome this misconception by adopting a comprehensive inquiry-based unit developed by the Appalachian Math Science Partnership (AMSP) that dealt with force and motion. The unit, developed by a group of college faculty and practicing elementary, middle, and high school teachers, was designed to use the constructionist approach developed by the McDermott group at the University of Washington, i.e., previous work was used as building blocks to increase the future understanding of

Table 5. Percentage of Students Correctly Answering Question 4.

School	Grade	Correct Responses
One	7	71.6
	7*	82.0
	8	42.6
	8*	94.5
Two	7	63.6
	8	67.5
Three	7	70.4
All	7	69.0
	8	70.0

Note. *classes receiving inquiry-based instruction.

student material. Note: The units are available through the University of Kentucky.

Our data also show that males outperform females in question one by more than 25 percentage points on average. This finding is in agreement with other works that compared gender performance in middle school science (Lee and Burkam 1998).

Question 2

Table 3 shows the results of Question 2 by grade level and school. It also includes a summary of the results for the entire group. The concept of acceleration is very complicated; therefore, we did not expect students to have a good understanding of it. We did, however, expect that at students were told that, "acceleration due to gravity is the same for all objects." We believed that students would answer this question correctly based on rote memory alone. A review of the student interviews, however, found that Question 2 had the lowest overall number of correct answers for all four questions, with only 27.2% of seventh graders and 21.7% of eighth graders choosing the correct answer. This indicates that students do not have a solid understanding of free-fall acceleration. We found that particular response patterns were dependent on the school where students attended.

The inquiry-based classes at School One had the best correct response rate, with 61.5% of seventh graders and 49.9% of eighth graders choosing the correct response. Conversely, School Two had a particularly low response rate, with 19.5% of seventh graders and 20.2% of eighth graders choosing the correct answer. School Two had more than 60% of seventh grade students choosing answer "a" and more than 74% of eighth grade students choosing answer "a." School 3 demonstrated a similar, though not as drastic, response rate to Question 2. We expected to find similar results to Question 1, which was the same question essentially, but phrased in everyday language. The responses to Question 1 and Question 2, however, were different in all cases, with a high percentage of students at all levels choosing the correct answer for Question 1 compared with Question 2. Our data also found that males scored higher than females on Question 2, and seventh graders

Table 6.   Percentage of individual answer choices.

School	Question	Grade	A	B	C	D
1	1	7	23.4	11.0	55.8	9.1
1		8	11.0	17.1	74.4	31.7
2		7	17.8	11.7	17.8	52.8
2		8	8.3	12.3	22.1	63.7
3		7	17.4	12.0	38.0	32.6
1	2	7	20.1	19.5	39.6	24.7
1		8	29.3	8.5	36.6	25.6
2		7	61.7	14.0	17.8	6.5
2		8	74.5	5.4	15.7	4.4
3		7	40.2	17.4	28.3	13.0
1	3	7	61.7	7.1	13.6	17.5
1		8	63.4	9.8	14.6	12.2
2		7	67.8	5.6	12.1	14.5
2		8	45.1	16.7	20.6	17.6
3		7	35.9	21.7	20.7	19.6
1	4	7	66.2	8.4	12.3	13.0
1		8	67.1	13.4	7.3	11.1
2		7	52.8	8.9	10.7	27.6
2		8	56.6	6.8	12.7	23.9
3		7	56.5	6.5	15.2	19.6

scored higher than eighth graders on Question 2. Our results suggest that students in middle-grade levels do not have a good understanding of acceleration and, specifically, not with regard to the context of free-fall.

Question 3

Table 4 shows the results of Question 3 by grade level and school. The table also includes a summary of the results for the entire group. The students had a better understanding of this question overall, with 66.1% of seventh graders and 57.0% of eighth graders choosing the correct response. The results were highest in the inquiry-based classes in School One, where 94.7% of the students answered the question correctly. In contrast, the students who performed poorly were the non-inquiry students at School One, particularly the eighth grade classes. We noticed a trend between seventh grade students who performed well on Question 3 and their eighth-grade counterparts. There was marked difference in the School One non-inquiry based classes, with seventh grade students outperforming their eighth grade counterparts by 25 percentage points. By comparison, the gap between the two grade levels in the inquiry-based classes was much smaller, with eighth graders tending to score better.

Students who chose an incorrect answer for Question 3 did not show a strong, common misconception. In School One and School Two, seventh graders who chose the wrong answer chose answer "d" most frequently while the eighth graders chose answer "c" more frequently. This suggests that the eighth graders at least thought there was a way to answer the question compared with the seventh graders who thought there was no way to know the answer. The students at School Three showed no strong preference for the incorrect answers, with only 35.9% of the students choosing the correct answer. This suggests that these students did not have a strong understanding of the concept of inertia. We were surprised to learn that more students did not choose answer "b," the probe would coast to a stop. We expected the students to think that if there were no forces acting on an object it will stop moving. Again, males tended to score higher than females on this question.

Question 4

We designed Question 4 to test the students' understanding of the difference between mass and weight. Table 5 shows the results according to grade, school, and class, as well as the entire group. Question 4 showed the best overall results with 69.0% of seventh graders and 70.0% of eighth graders choosing the correct answer. We continued to see a trend among students receiving inquiry-based instruction and high scores. The poorest

results for this question came from eighth grade students in School One who received traditional instruction. Again, we noticed a trend among students receiving inquiry-based instruction and high scores. Seventh grade students performed better than their eighth grade counterparts with the noted exception among eighth graders at School One who received inquiry-based instruction.

The most commonly chosen incorrect answer for Question 4 was *d*, i.e., both your mass and weight decrease." This suggests that the students are aware that you would weigh less on the moon and think that you must have less mass for that to be true. We also noticed that males tended to perform better than females; however, the difference between the genders was only about five percentage points.

STUDENT INTERVIEWS

We recorded video interviews of eighteen students from School One and then transcribed them afterward. The interviewer asked each individual a core set of questions. We asked some students follow-up questions to clarify their ideas, if necessary. The interviewer took care not to lead the students or provide information or clues about the correctness of the answers or explanations. We interviewed a mixture of seventh and eighth grade students who were chosen randomly by their teachers. We conducted the interviews at the end of the spring term, about six weeks after the students had completed the unit in their science class on force and motion. We included students who had completed the inquiry-based unit as well as some students who had taken a traditional class. We divided the interview questions into four sections based on content: 1) free-fall, 2) force-inertia, 3) mass/weight, and 4) acceleration.

Free-fall

At the start of the interview, we gave each student two identical sheets of paper. We asked the students to describe the paper, and then we quizzed them until they indicated that both papers were the same size and weight. Next, we told the students to crumple one sheet of paper, lift both sheets of paper to the same height, and predict what will happen if both pieces of paper were dropped at the

Table 7. Students' Ideas on Free-fall Questions.

	n	%
Q1. Why did the crumpled paper fall faster?		
a. Crumpled paper is heavier	7	38.9
b. Air kept flat sheet up longer	8	44.4
c. Crumpled paper is more dense	1	5.6
d. Crumpled paper has lower mass	1	5.6
e. No answer	1	5.6
Q2. What will happen when the two balls are dropped?		
a. Heavier one will hit first	16	88.9
b. They will hit at the same time	2	11.1
Q3. Why did they fall at the same rate		
a. Same size	2	11.1
b. Same density	1	5.6
c. Force of gravity is the same	4	22.2
d. Because they fall at the same time	1	5.6
e. No answer/Don't know	4	22.2
f. Denied Observation	6	33.3

same time. We did this to familiarize the students with the difference between regular-fall and free-fall. We then asked the students to drop both pieces of paper and describe the results.

We then asked the students to inspect two equal-sized balls with radically different weights. We asked the students to describe the balls, and then we quizzed them until they stated the balls were of the same size but of different weights. We did not lead the students to this conclusion; however, it was important that that they understood that the size of the balls was not a variable in free-fall motion. We chose balls that were of small enough to neglect air resistance. We then asked the students what would happen if the balls were dropped from the same height at the same time. The students then dropped the balls to test their hypothesis.

We asked them the following questions during the interview:

Q1.   Why did the crumpled paper fall faster?
Q2.   What did they expect would happen when the two balls were dropped?
Q3.   Why did the two balls fall at the same rate?

We included a summary of the students' responses in Table 7. We asked the students these questions regardless of whether or not their predictions were consistent with the observed results.

Table 8.   Students' ideas on force-inertia questions.

	n	%
Q4. What is a force?		
a. Push or Pull	8	44.4
b. Moving object	2	11.1
c. Ability to move and object	1	5.6
d. Amount of pressure put on an object	1	5.6
e. No answer/Don't know	5	27.8
f. "Something that makes something do something"	1	5.6
Q5. After your hand is removed, what did ball do?		
a. Slows down	8	44.4
b. Constant speed	8	44.4
c. Speeds up	2	11.1
Q6. What keeps the ball moving?		
a. Force of my hand	7	38.9
b. Unknown force	4	22.2
c. Gravity	2	11.1
d. Weight	2	11.1
e. Inertia (but could not define it)	2	11.1
f. Force of acceleration	1	5.6

Our expectations were that students would answer that air resistance, friction, or a parachute-type effect would make the flat piece of paper fall slower than the crumpled piece of paper. This was not the case, however, as many students used the model of heavier objects falling faster than lighter objects. To fit their observations into this model 38.9% of the students indicated that crumbling paper made it heavier. This was a new misconception, so we conducted a literature review to see if it had been observed previously. These findings suggest that students work from models that they consider intuitive.

The two-ball experiment supported the intuitive model idea. An overwhelming majority of the students (88.9%) predicted that the heavier object would fall faster than the lighter object and, thus, would hit the table first. We found another interesting result in this section of the interview, too. Thirty-three percent of the students who dropped the balls, sometimes repeatedly, refused to acknowledge that the balls hit the table at the same time. This is consistent with previous work performed by Gunstone and White (1981), who found that first-year college students were likely to observe the prediction they made regardless of the actually outcomes.

The responses to Question 1 on the written test were similar to the responses to Q2 from the interviews. On the written test, 9.1% of seventh graders and 31.7% of eighth graders from School One incorrectly answered Question 1, choosing option "d" as their answer, i.e., heavier objects fall faster than lighter objects, was only. In contrast, 88.9% of the same respondents chose this answer in the interviews. This finding shows how difficult it is for students to overcome misconceptions in science.

Force-Inertia. For this section of the interview, we asked students to describe a force. They were given a ball and asked to roll it across a table that had very little friction. We then asked them to describe the ball's motion while their hand was on the ball and to describe ball's motion after their hand was removed. We asked them the following questions:

Q4.   What is a force?
Q5.   When you removed your hand from the ball, what did it do? speed up, slow down, or move at constant speed?
Q6.   What keeps the ball moving after your hand was removed?

Table 8 provides a summary of the students' answers to the force-inertia questions. On Q4, nearly half (44.4%) of the students were able to correctly describe a force. The one student who gave the answer, "Something that makes something do something," is interesting because it seems like the student has the correct idea about forces, but is incapable of correctly verbalizing that idea. The results for Q5 show the difference between what students observed and what they predicted. We found that 44.4% of the students believed that the ball slows down after being released, even though care was taken to ensure them that the ball was moving at constant speed. The students expected the ball to slow down given their prior experience with a rolling a ball on the ground. This differs from the results of Question 3 on the written test, which dealt with the motion of a spacecraft. In this case, the students did not show the expected misconception. Our results, supported by Q6, showed that students did not understand that the concept behind these two different situations could be in one word: inertia.

Table 9. Students' ideas on mass/weight questions.

	n	%
Q7. What is weight?		
a. Amount of mass	7	38.9
b. How heavy an object is	3	16.7
c. Gravitational pull on object	4	22.2
d. Size	1	5.6
e. Weight	3	16.7
Q8. What is mass?		
a. How heavy an object is	7	38.9
b. How dense an object is	1	5.6
c. How much there is of something	1	5.6
d. Size	4	22.2
e. How much matter is in an object	4	22.2
f. Don't know	1	5.6
Q9. What happens to your weight on the Moon?		
a. It decreases	17	94.4
b. You are weightless on the Moon	1	5.6
Q10. What happens to your mass on the Moon?		
a. Same	15	83.3
b. Increases	1	5.6
c. Decreases	2	11.1

Table 10. Students' ideas on acceleration questions.

	n	%
Q11. What is acceleration?		
a. Speeding up but not slowing down	7	38.9
b. Speeding up or slowing down	4	22.2
c. Speed	7	38.9
d. Force making an object move	1	5.6
Q12. Is car moving at 60 accelerating more than car moving at 30 mph?*		
a. Yes, more acceleration at 60 mph	9	69.2
b. No, but could not explain	2	15.4
c. No, gave good explanation	1	7.7
d. No, it depends on how far you push the pedal down	1	7.7

Note. *Only 13 of the 18 students were asked this question.

On Q6, two of the students stated correctly that inertia kept the ball moving after they let it go; however, neither of the students was able to describe what they meant by "inertia." This suggests that they were just memorizing something their teacher told them or something they read in their textbook. We found this especially disturbing because inertia is a fundamental concept of motion and free fall, and it is difficult to understand them without first understanding the concept of inertia. A majority of students (61.1%) believed that there was a force that caused the ball to remain in motion, and that the force was applied by their hand or some unknown force. This is consistent with previous works by Clement (1982) and Galili and Bar (1992), who showed that students believe that motion implies force.

Mass/Weight. We asked the students the following questions about mass and weight.

Q7. What is weight?
Q8. What is mass?
Q9. What happens to your weight if you are moved from the Earth to the Moon?
Q10. What happens to your mass if you are moved from the Earth to the Moon?

The summary of answers for the mass/weight questions are shown in Table 9. The answers from Q7 and Q8 show that most of the students were incapable of defining weight or mass. A few of the students said that weight is "how heavy an object is." This is another example showing how sense of experience was applied to learning; however, the students had no actual understanding of how to define weight and mass scientifically. Many students gave circular definitions of weight was mass and then said mass was weight. All but one student who answered Q9 knew that you would weigh less on the Moon; yet, 83.3%, correctly answered that your mass would be the same on the Moon as it is on Earth. The students performed better on Q9 and Q10 in the interviews than they did on the Question 4 of the written test, which covered similar content. Furthermore, more students answered Q9 than they did any of the other interview questions.

Acceleration. In the final section of the interview, We asked the students two questions about acceleration:

Q11. What is acceleration?
Q12. Is a car moving at 60 mph accelerating more than a car at 30 mph?

Table 10 summarizes the answers to the acceleration questions. Only 22.2% of the students gave a satisfactory definition of acceleration stating that accelerate refers to "speeding up or slowing down." A greater number of students said that acceleration was, "speeding up, but not slowing down," which most likely comes from the common usage of the term acceleration used in relation to automobiles speeding up. Seven

of the students (38.9%) said that acceleration was speed, which is consistent with the answers to Q12 where 69.2% of the students asked stated that a car moving at 60 mph is accelerating more than a car moving at 30 mph.

Summary of Student Interviews.   Overall, the interviews showed that students work from intuitive models and are reluctant to deviate from those models to explain observations in science. For example, the students thought that heavier objects fall faster than lighter objects. To explain their predictions that a crumpled paper wad will fall faster than an uncrumpled sheet of paper, the students must formulate a model in which the crumpled paper weighs more than the uncrumpled paper. Their intuitive models led to predictions that, in some instances, were absurd, i.e., the act of crumpling paper mad it heavier. Their intuitive models also caused them to deny direct evidence, such as the demonstration involving the two balls of same size with different masses.

The observations from this study, coupled with the student's inability to formulate concepts of inertia and their lack of understanding of what causes free fall, combined with their lack of a vocabulary of basic terms, such as mass, weight, and acceleration, lead to erroneous models. For example, many students stated that the reason the two balls fell at the same rate was that the pull of gravity was the same on both objects. We do not believe they would have said this if they understood that weight is the pull of gravity. In the case of free fall, the students had not learned the reasons behind free fall and did not have the concepts or language to formulate reasoned responses. Some students, who first predicted that the heavier objects would fall faster than the lighter objects, corrected themselves and said "No, wait, we did this in class." It became clear that doing or telling alone was not going to address student misconceptions in science. Instead, an understanding must be included in the discussion.

This indicated to us that just telling the students or demonstrating a principle for them was not sufficient instruction unless it was followed up with sound scientific principles that included the phenomenon they just witnessed, such as Newton's Laws. In addition, teachers must include the concepts that are necessary to formulate reasoned responses, such as acceleration or inertia, in their discussion on free fall.

DISCUSSION

The performance of seventh and eighth grade students when addressing a simple set of questions related to free fall, force-inertia, and acceleration showed a lack of understanding about the nature of these quantities and a lack of ability to explain these phenomena in terms of simple, fundamental quantities, and laws. One notable exception was the inquiry-driven unit on force and motion that were selected for classes at School One. We found that students who were members of the class using these techniques performed better than students in other classes did. The inquiry-based unit developed by AMSP stressed that reasoning for observations, such as free fall, needed to be incorporated into the unit's structure. While it is known that interactive instruction, such as inquiry, is superior to traditional instruction (Hake 1998), in this case it was not enough by itself. Our data clearly show that students will deny observations that conflict with their intuitive models. "Finishing the deal" in class is important because it relates to summarizing simple laws and concepts that students can use to explain observations.

Our data also highlighted performance differences among students that depended on which school the students attended. Overall, the poorest results were seen in School Two, which also had the highest percentage of students who received free lunches (see Table 1), which indicated that it had the highest low-income population among the three schools in the study.

Overall, the results from the written test and the interviews showed that the students had a poor understanding of free fall, force/inertia, mass/weight, and acceleration. We also found little or no added gain when we compared seventh grade students to eight grade students. In fact, the seventh graders outperformed the eighth graders on three out of the four written questions. Our data also showed that the males outperformed the females on all four questions. These findings show that there was considerable room to

enhance students' understanding of these topics. Furthermore, we found evidence that eighth grade students who received inquiry-based instruction significantly outperformed than their peers, which corroborates earlier studies' that found success was related to the method of course delivery.

FURTHER STUDY

We plan to continue our pre- and post-unit student analysis of students who participated in School One's inquiry-based unit that on force and motion. We plan to compare these students with other students who were taught using the traditional lecture mode of teaching. Currently, we are analyzing these results for a later presentation. We plan to present data, at a later date, from the video interviews we conducted at two of the middle schools along with more gender comparisons that were based on those interviews.

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Comparison of Remotely-triggered Cameras vs. Howling Surveys for Estimating Coyote (*Canis latrans*) Abundance in Central Kentucky

Tyler W. Morgan and Charles L. Elliott[1]

Department of Biological Sciences, Eastern Kentucky University, Richmond, Kentucky 40475
KEY WORDS: Camera Survey, Coyote, *Canis latrans*

A common problem associated with research efforts for wide-ranging predators is the estimation of population abundance. While mark-recapture studies incorporating live trapping can yield reasonable estimates, these techniques are often labor intensive and cost prohibitive, and introduce behavioral biases into sampling efforts. As such, noninvasive sampling efforts are increasingly being used to census predator populations in an effort to provide more robust and logistically feasible indices of abundance. We studied coyotes (*Canis latrans*) on the Blue Grass Army Depot in central Kentucky from 2007 to 2008 to evaluate two noninvasive sampling techniques for estimating population abundance. Motion-sensitive, remotely-triggered cameras and howling surveys were concurrently used to census coyotes on the study area. We recorded 2698 photographs and identified 8 coyotes on the study area; while howling surveys documented a maximum of 13 individuals. We detected no significant difference ($P < 0.05$) in mean coyote abundance based on the minimum (Rank Sum Test, T = 20.5) and maximum (T = 20) number of coyotes detected between camera and howling surveys. We recommend that howling surveys be used to estimate coyote abundance in areas accessible by motorized travel; and camera surveys be employed in more remote areas where vehicular traffic is not feasible.

Coyotes (*Canis latrans*) have been studied extensively in North America, With particular research interest focused on means to monitor and regulate their populations (Schemnitz 2005). Historic noninvasive survey techniques provide indices of coyote abundance, e.g., track and scat surveys (Heinemeyer et al. 2008; Ray and Zielinski 2008); howling surveys (Andrews 1979; Wilson and Delahay

2001); scent stations (Harrison 1997, 2006); trends in trapper reports (Wemmer et al. 1996). In addition, more intensive noninvasive studies that provide actual estimates of abundance include radio telemetry (Wemmer et al. 1996); and endocrine monitoring and genetic sampling (Kendall and McKelvey 2008; Schwartz and Monfort 2008).

More traditional procedures for assessing coyote populations, such as scent stations (Wemmer et al. 1996), provide only indices of relative abundance that may not be sufficiently accurate or robust for some aspects of management; especially considering that visitation rates can be low (Harrison 1997). Although radio telemetry and mark-recapture studies can yield estimates of animal density (Southwell 1996), the techniques necessitate fiscal, temporal, and logistical requirements that are often not feasible for wildlife agencies. Those constraints are often compounded when considering research efforts involving wide-ranging species that have low recapture probabilities (Heilbrun et al. 2006). With traditional studies involving the live capture of animals coming under increasing scrutiny (Schemnitz 2005), wildlife investigators are resorting to less invasive sampling methods to evaluate the population status of free-ranging wildlife. More cost-effective and less invasive methods, such as remotely triggered cameras ('trail' cameras), have yielded positive results and may provide a viable alternative for many wildlife researchers (Moruzzi et al. 2002). Evaluating the use of remotely triggered cameras to survey carnivore spatial arrangements, Moruzzi et al. (2002) acknowledged the difficulty of studying carnivores and the utility of cameras for detecting elusive, nocturnal, or secretive species.

Remotely-triggered cameras have been used as a research tool to study aspects of avian ecology (Franzreb and Hanula 1995;

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[1] To whom correspondence should be addressed. E-mail: charles.elliott@eku.edu

Swann et al. 2004; Staller et al. 2005), individually identify predators (Heilbrun et al. 2003; Mendoza et al. 2011), describe predator feeding characteristics (Savidge and Seibert 1988; Hegglin et al. 2004), census big game species (Koerth et al. 1997; Roberts et al. 2006; McCoy et al. 2011), and estimate density of secretive species (Dobey et al. 2005; Harrison 2006; Symmank et al. 2008; Bengsen et al. 2011; Foster and Harmsen 2012). Those studies illustrated the utility of remotely-triggered cameras and highlighted the inadequacy of other methods that do not use cameras (Sanderson and Trolle 2005; Staller et al. 2005). Population studies in the southeastern U.S. that have employed remotely-triggered camera systems are relatively few. Roberts et al. (2006) conducted a study of Florida Key deer (*Odocoileus virginianus clavium*) comparing road survey estimates to mark-resight estimates derived from infrared-triggered cameras and found that cameras provided significantly higher population estimates. In Georgia, Holtfreter et al. (2008) determined the minimum length of time cameras must be deployed to attain sufficient detection probabilities for three classes (adult sows, adult boars, juveniles) of feral pigs (*Sus scrofa*). Martorello et al. (2001) used a mark-resight technique to estimate North Carolina black bear (*Ursus americanus*) population size by using bait-triggered cameras and indicated cameras provided less expensive, more accurate population estimates. Symmank et al. (2008) concluded the less intrusive nature of remotely triggered cameras yielded a safer environment for both wildlife and researchers when compared to survey methods involving the live capture of animals. Likewise, the reduction in time spent in the field makes remotely-triggered cameras a more cost-effective means of sampling in relation to other methods used to estimate population size (Symmank et al. 2008). In light of the less invasive nature of remotely-triggered cameras, researchers have identified issues that may introduce biases into the sampling method. Larrucea et al. (2007) noted human activity and scent, and the presence of camera equipment may conflict with the underlying assumption of equal catchability associated with most survey methodologies.

Due to the difficulty and time required to adequately sample wide-ranging carnivores, methods that are logistically simpler and less time-consuming are needed. Remotely-triggered cameras may be one such method because the technique provides photographic documentation of the targeted species (Jacobson et al. 1997). To-date, there have been no published studies using remotely-triggered cameras to estimate abundance or density of coyotes in the southeastern U.S. The objective of this study was to assess the usefulness of remotely-triggered cameras in estimating coyote abundance in east-central Kentucky by comparing the abundance estimates obtained from concurrent camera and howling surveys.

MATERIALS AND METHODS

The study was conducted on the Blue Grass Army Depot (BGAD), a 59,076 ha tract of federally-owned land located 11 km south-southeast of Richmond, Madison County, Kentucky. The BGAD is comprised mainly of open fields and forested woodlots that range in size from 1 to 50 ha. The BGAD is a multiple-use military installation which incorporates grazing, hay production, silviculture, and management of wildlife resources as part of its integrated natural resources management plan (Stout et al. 2005).

Ten remotely-triggered digital cameras (Game Spy 200; Moultrie Feeders, Inc., Alabaster, AL) were deployed from May 2007 to May 2008. Each camera was equipped with a passive-infrared sensor, with a range of approximately 10 m, to detect movement and trigger the camera. Cameras had rechargeable batteries equipped with solar trickle-chargers. Each photograph obtained was individually identified with the time, date, temperature, moon phase, and location.

Crossett (1990) found that the highest mean hourly movement of coyotes (0.39 ± 0.36 km/hr) in areas near the BGAD occurred during the dispersal period (from August through December). Based on Crossett's (1990) mean hourly movement rate, and our desire to be able to segregate photos taken within a one hour time period on any sampling day, cameras were placed ≥1.5 km apart to minimize the possibility of double sampling (photographing the same coyote at two different camera stations within the same

hour). Using a land cover map of the BGAD in association with GIS software, the BGAD was divided into a series of circles (sampling units) with radius equal to 0.75 kms.

Ten sampling units were randomly selected as camera locations. We followed the recommendations of Swann et al. (2004) and attached cameras to very firm supports (i.e., tree trunks) approximately 40 cm above the ground. When necessary, natural vegetation in the foreground of the photographic plane was removed so as to not inadvertently trigger the camera.

In their review of the use of remote cameras as a carnivore survey method, Kays and Slauson (2008) noted that once a sample unit is selected, cameras are typically placed in areas which are frequented by the target species or at stations with attractants designed to lure the animal to the camera. Coyotes on the BGAD have been observed using roads and stream corridors as travel routes (C. Elliott pers. observ.). From the center point of each sampling unit, all cameras in this study were located ≤10 m of a stream corridor or road. Although Larrucea et al. (2007) reported no difference in photo-capture success of coyotes between camera sites with and without scent attractants, we employed the use of a scent attractant in the hope of enhancing our photo-capture success rate. Camera sites were baited with fatty acid scent (FAS) discs (U.S.D.A Supply Depot, Pocatello, ID) and placed 2–5 m from the bait. Cameras were programmed to record activity on a continual basis (Larrucea et al. 2007). We collected photographs from each camera, and replenished FAS discs, every 7 days. Upon each visit, photographs were transferred from 256-megabyte memory cards to a laptop computer and memory cards replaced.

We conducted six howling surveys (Harrington and Mech 1982) between October 2007 and April 2008. The howling survey route was static and consisted of 10 calling stations located 1.6 km apart. The survey route bisected the area in which cameras were located. Howling surveys were conducted at night using a commercially available electronic caller (Johnny Stewart Preymaster Digital Call; Hunter Specialties, Cedar Rapids, IA). Surveys commenced at 2200 hours and the calls of coyotes were broadcast

Table 1. Summary of photographs obtained from remotely-triggered cameras and howling survey results, Blue Grass Army Depot, Madison County, Kentucky.

Summary of Photographs (May 9, 2007–May 14, 2008)	
Item Photographed	Number of Individual Photographs
White-tailed Deer (*Odocoileus virginianus*)	1064
Domestic Cattle	540
Eastern Turkey (*Meleagris gallopavo*)	109
Coyote (*Canis latrans*)	8
Fox Squirrel (*Sciurus niger*)	3
Raccoon (*Procyon lotor*)	2
Bobcat (*Lynx rufus*)	1
Eastern Cottontail (*Sylvilagus floridanus*)	2
Unknown animal (could not be clearly identified)	32
False triggers (camera triggered by floodwater or wind)	937
TOTAL	2698

Howling Survey Results

Date of survey (minimum and maximum number of individuals heard)

3 October 2007 (5 to 6 individuals)
27 January 2008 (2 to 3)
2 March 2008 (4 to 6)
11 March 2008 (0)
12 April 2008 (3 to 5)
14 April 2008 (11 to 13)

for 3 minutes at each calling station. We recorded the maximum and minimum number of responsive coyotes for a period of 5 minutes. Professional judgment was used to eliminate responses that were suspected to be from domestic dogs rather than coyotes. Howling surveys were not conducted when windy or rainy conditions prevailed. The rank sum test (Ambrose and Ambrose 1987) was used to identify any significant difference ($P < 0.05$) in mean coyote abundance based on the minimum and maximum number of coyotes detected between howling compared to trail camera surveys.

RESULTS

From 9 May 2007 to 14 May 2008, a total of 3234 camera-trap nights were accumulated from 10 camera sites. During the course of the study, 3 cameras became inoperable and were not replaced. Collectively 2698 photographs were recorded; 1761 were of vertebrates (Table 1) while 937 were considered "false"

triggers as no vertebrates were observed. Of the coyote photographs recorded, five were obtained between mid-December 2007 and mid-February 2008, while seven of eight were obtained between the latter part of the dispersal and beginning of pup rearing seasons [December to May; Crossett (1990)]. Howling surveys indicated a maximum coyote abundance of 13 individuals present during the study (Table 1). We failed to detect a significant difference in mean coyote abundance based on the minimum (T = 20.5) or maximum (T = 20) number of animals detected for howling surveys versus camera results.

DISCUSSION

The technique of using remotely-triggered cameras to estimate coyote abundance or density tends to suffer from low photo-capture success rates [see review by Kays and Slauson (2008)]. In this study, photographic success rates were considerably lower than those observed in similar studies (Gompper et al. 2006; Larrucea et al. 2007). Larrucea et al. (2007) noted that technical issues such as the geographical and temporal placement of cameras can subsequently influence sampling success and level of introduced bias. Specific characteristics of individual sampling locations, such as amount of local human activity (i.e., scent), proximity to roads or other travel ways, and habitat types, have all been reported to influence photo-capture success (Larrucea et al. 2007).

To photo-successfully sample (i.e., photograph) coyotes as randomly as possible researchers must consider seasonal and daily activity patterns (Larrucea et al. 2007).

On areas where coyote densities are low, we suggest that cameras be allowed to operate continuously across all seasons. Likewise, we suggest that camera locations cover an area large enough to ensure capture probabilities that will yield useable data. We also suggest that prior to sampling, cameras be left at each camera location long enough to allow wary animals acclimate to them (Larrucea et al. 2007).

Territorial distribution and home range dynamics largely influence where coyotes will be successfully photographed (Sequin et al. 2003). Sampling efforts concentrated in pre-ferred habitats may record high photo-capture rates of a large proportion of the local coyote population, but that collective sample could be biased towards resident familiar units (Larrucea et al. 2007). Likewise, concentrating sampling efforts in less preferred habitats may yield data biased towards dispersing males, transient coyotes, or individuals on the periphery of their existing territory (Larrucea et al. 2007). As such, the temporal component of photographic sampling should be given equal consideration as this factor can help overcome biases inherent to efforts that are limited in geographic scale. Camera success in this study was highest during the spring, a trend also noted by Larrucea et al. (2007) in California. Larrucea et al. (2007) postulated many young adult coyotes disperse in the spring and travel great distances through unfamiliar areas, making them more vulnerable to being photographed.

The use of baits or attractants to entice target species to visit a specific location is a time-tested procedure (Sumner and Hill 1980; Linscombe et al. 1983; Kimball et al. 2000). Gompper et al. (2006) used both baits and olfactory attractants, but did not report a higher photo success rate than Larrucea et al.'s (2007) study that did not incorporate bait into the sampling design. The use of FAS has been reported to vary in its success as a lure for attracting furbearers (Sumner and Hill 1980). The success rate in attracting coyotes to camera traps in this study may have been improved if another scent lure had been employed.

The BGAD contains 245 km of paved and gravelled roads, as well as 65 km of railroad rights-of-ways (Stout et al. 2005). Linear features such as these hamper prediction of coyotes' travel routes, rendering systematic placement of cameras less effective for detecting the animal. We believe the abundance of human travel corridors for coyotes on the BGAD had a major impact on our sampling success and the number of coyote photographs recorded. In their study assessing the influence of various factors on coyote vulnerability to photo-capture, Larrucea et al. (2007) observed coyotes were more likely to be captured (photographed) at stations placed on roads, while pups were more likely to be photographed at non-anthropogenic sites.

Howling surveys, the broadcasting of calls of conspecifics to elicit vocal responses, have been used to determine the abundance of a number of canid species (Morse and Balser 1961; Harrington and Mech 1982; Fuller and Sampson 1988). Conroy (1996) discussed howling surveys and noted that a key assumption associated with auditory indices is that the index bears the same relationship to abundance at each sampling location or occasion. Work by Harrington and Mech (1982), Fuller and Sampson (1988), and Laundre and Keller (1984) suggests that call rates of wolves (*Canis lupus*) and coyotes are influenced by behavioral and environmental factors such as daily and seasonal social dynamics, animal movement, weather and lighting conditions. In addition, variations in topography and vegetative cover can greatly affect the radius within which animals or observers respond to or detect calls (Harrington and Mech 1982).

The objective of this study was to assess the usefulness of remotely-triggered cameras in estimating coyote abundance in east-central Kentucky by comparing results of a camera-survey with those from concurrent howling surveys. Collectively, it is our assessment that howling surveys provided the most economical and cost-effective survey technique for providing an index of abundance for coyotes on the BGAD. We recommend that researchers consider the level of human access and location of anthropogenic corridors in a region (i.e., foot trails and roads) when selecting between howling or camera surveys for estimating the abundance of coyotes at sites in Kentucky. In areas where human access is mainly by foot trails, we recommend employing camera surveys in the Spring months to create an index of coyote abundance. Coyotes would most likely use the trail system as movement corridors, hence camera "trap" sites baited with a species-specific lure would probably produce the greatest number of photographic captures. Even though coyotes have been found to be more likely photographed at stations placed on roads (Larrucea et al. 2007), our results suggest that surveys may be the most effective procedure to use in areas with moderate-to-heavy road densities.

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Micropropagation, Cryopreservation, and Outplanting of the Cumberland Sandwort *Minuartia cumberlandensis*

Valerie C. Pence,[1] Bernadette L. Plair, Susan M. Charls, and John R. Clark

Center for Conservation and Research of Endangered Wildlife, Cincinnati Zoo and Botanical Garden, 3400 Vine Street, Cincinnati, Ohio 45220

and

David D. Taylor

Daniel Boone National Forest, 1700 Bypass Road, Winchester, Kentucky 40391

ABSTRACT

Cumberland sandwort (*Minuartia cumberlandensis*) is a federally endangered species native to Kentucky and Tennessee. An in vitro propagation protocol was developed for producing plants for potential outplantings to increase numbers in the wild. Cryopreservation protocols were developed to preserve in vitro lines ex situ. Initial survival of shoot tips through liquid nitrogen exposure ranged from 62–94%, while survival after 10 years in LN ranged from 10–75%. An experimental outplanting of 63 in vitro-propagated plants was undertaken in a rockhouse habitat within the historic range of the species. After 6 years, 12 of the original plants were surviving, but the population had grown to over 200 plants. Survival varied within the rockhouse and appeared to be related to moisture and light variations at the different microhabitats within the site. These results demonstrate that in vitro propagation methods can be used to supplement other conservation efforts with *M. cumberlandensis*, by supplying plants for outplantings, if needed. In vitro propagated plants can also be used to study microhabitat requirements and to select locations to increase survival of outplanted individuals, without depleting the genetic diversity available for reintroductions.

KEY WORDS: Micropropagation, Reintroduction

INTRODUCTION

Minuartia cumberlandensis McNeill (Cumberland sandwort, Cumberland stitchwort, Caryophyllaceae) (*Arenaria cumberlandensis* Wofford and Kral) was described in 1979 as a new species from the Cumberland Plateau (1). It grows in sandstone rockhouses, a wetter, shadier, habitat than other closely related sandworts. It is known from likely fewer than 100 occurrences, all within 25 miles of each other in southern Kentucky and northern

Tennessee. *M. cumberlandensis* is federally listed as endangered and is threatened by hiking, rappelling, and digging for artifacts (2, D. Lincicome).

Because of the small numbers of plants of this species and the vulnerability of some of the small populations, in vitro methods were explored to determine their effectiveness in propagating *M. cumberlandensis* and providing plants that would survive in potential augmentation or reintroduction projects. The following studies were undertaken to: a) develop a tissue culture propagation protocol for this species; b) develop a cryopreservation protocol for in vitro tissues; and c) examine the ability of tissue culture-propagated plants to survive outplanting in a natural setting.

MATERIALS AND METHODS

Initiation and Propagation of Cultures

Seeds of *M. cumberlandensis* were sent to CREW from the Missouri Botanical Garden, from seed lots 91149 and 942375 collected at

Hazard Cave and Twin Arches Trail, respectively, at Pickett State Park, Fentress Co., Tennessee. For germination, seeds were surface sterilized in a 1:20 dilution of commercial bleach with 0.05% Tween 20 for 5 min, followed by two rinses in sterile, pure (reverse osmosis) water. Seeds were then placed either onto 1% agar alone, or onto half-strength Linsmaier and Skoog (3) medium (LS medium) with 1.5% sucrose and 0.22% gellan gum in 60 × 15 mm disposable plastic Petri dishes, 15 ml medium/dish, 5 seeds per plate. Seeds were incubated under three conditions: 1) 26°C with a 16:8 hr light:dark cycle, at approximately 20 μmol/m²/s photosynthetically active radiation (PAR) (2) in a variable temperature incubator with a 16:8 hr 30°C:15°C, light:dark cycle with light of similar intensity; or 3) at 4°C in the dark. The resulting seedlings were then used to initiate shoot propagating cultures by excising the shoots and transferring them to full-strength LS medium with 3% sucrose and 0.22% Phytagel, plus either 0.5 mg/L benzylaminopurine (BAP) and 0.05 mg/L naphthaleneacetic acid (NAA) or 0.1 mg/L BAP and 0.01 mg/L NAA. Lines initiated with the higher levels of growth regulators were moved to the latter medium after one or two subcultures, and that medium was used for maintaining the cultures (propagation medium). Woody Plant (WP) medium (4) with and without 0.5 mg/L indolebutyric acid (IBA) was used to initiate roots in vitro.

Rooted shoots were transferred from cultures to a soil mix of 2:1:1 parts sand: Pro-Mix:pine fines, approximately 3 cm deep, in 10 × 10 × 11.5 cm polystyrene culture boxes (Phytotech) or in trays in the greenhouse under mist. Box lids were slowly opened to acclimatize the plants to ambient conditions.

Cryopreservation of Shoot Tips

Shoot tips, approximately 1 mm in length, were isolated for cryopreservation from in vitro shoot cultures 46 to 114 days in age that had been grown on LS basal medium with 0.1 mg/L BAP and 0.01 mg/L NAA with 0.8% agar. Prior to isolation, shoots were cold hardened at 4°C for 7–14 days. After isolation, shoot tips were placed onto the same medium plus 0.3 M mannitol and 10 μM abscisic acid (ABA) with 0.22% gellan gum in place of agar, in 60 × 15 disposable Petri plates, 15 ml of medium per plate, for 48 hours. Shoot tips were then prepared for liquid nitrogen (LN) exposure using the encapsulation vitrification procedure (5). After LN exposure for at least 30 minutes, some shoot tips were thawed in a 38°C water bath, rinsed with a solution of 1.2 M sucrose, and transferred to plates of propagation medium. For recovery growth, shoot tips were incubated under the same temperature and light conditions used for culture maintenance. The remaining shoot tips were banked in long-term, liquid nitrogen storage in CREW's CryoBioBank,® with 10 shoot tips per vial.

Two vials each of four lines were removed after 9–10.5 years of storage in a Thermolyne Locator 4 LN storage tank equipped with four holders, nine boxes per holder, each box with 96 sample vials. Each holder contained multiple samples from different species, and the holder was removed quickly from the tank each time a new sample or group of samples was added or removed.

The samples of *M. cumberlandensis* that were removed from storage were rinsed and cultured for recovery using the same methods as for control samples that were removed from LN within an hour of the original banking. Viability of the control samples was used as the initial viability of the stored sample. In initial and long term samples, survival was measured as the number of shoot tips remaining green and showing growth at 3 weeks. For the samples stored long-term, an evaluation was made of the number of times each sample had been exposed to a potential "microthaw" event by being in a holder that had been quickly removed from the storage tank when new samples were added to the holder. Such removal would not be long enough to thaw samples, but would raise the temperature slightly for 15–30 seconds. Based on the dates of entry of other samples in the same holder, the number of potential "microthaw" events was calculated for each of the four samples.

Experimental Outplanting

On 27 August 2005, 63 acclimatized plants from seven genetic lines were planted at a site in McCreary Co., Kentucky, in the Daniel

Figure 1.   Site of *M. cumberlandensis* experimental outplanting, McCreary Co., Kentucky.

Boone National Forest (Figure 1). The site was a rockhouse formation within the natural range of the species, but not known as an historic site. Plants were planted in groups in seven locations within the rockhouse that differed in moisture and light levels (Table 1). Plants from each line were included at each site. The sites of the plants were labeled and mapped within the rockhouse. Each site was originally described qualitatively in terms of moisture and light (Table 1).

Plants were monitored at 2 months, (27 October 2005), and then during the years following planting: 6 July 2006, 27 July 2007, 25 July 2008, 25 August 2009, 27 July 2010, and 12 August 2011. In 2009, measurements of light, soil pH, temperature, and humidity were made at several of the sites within the rockhouse, to provide information for each site relative to other sites. In 2011, soil samples were taken from several of the sites to provide relative information on soil moisture on that date. Light was measured using a light meter (Fisher Scientific, #06-662-64); temperature and humidity were measured using a thermometer-hygrometer (Fisher Scientific #14-69-84), and

soil pH was measured by mixing water and soil (2:1 v/v) for 15 minutes, allowing the soil particles to settle, and measuring the pH of the aqueous phase (Fisher Scientific, Accumet Basic AB15 pH meter). Soil moisture was measured gravimetrically by weighing a soil sample, drying overnight at $100°C$, and re-weighing the sample. Percent moisture was calculated on a wet weight basis (FW $-$DW/ FW $\times$ 100) and averaged for triplicate samples.

Fourteen additional plants were planted between sites 4 and 5 in 2009 and 5 more added in 2010. These were not included in the totals, but soil moisture and pH were determined for this new site (Table 1).

RESULTS

Initiation and Propagation

When germination was tested, seed lot 942375 appeared to have higher viability (average 50%) than seed lot 911449 (average 12%) when germinated at a constant $26°C$ (Table 2). There appeared to be slightly better germination when variable or cold temperature treatments were used with lot 942375.

Table 1.   Descriptions and measurements of the planting sites for *M. cumberlandensis* within the rockhouse. Original site descriptions were made in 2005, soil moistures were measured in 2011. All other measurements were made in 2009.

Site (No. planted)	Original Site Description	Light (ft-cd)		Soil moisture %	pH	RH	Temp
		Cloudy	Sunny				
1 (12)	Site drier than most in shelter, but with moderate light	111–120	200–211	14.0	4.5	86–90	73–78
2 (10)	Site drier and darker than most in shelter	113–119	215–219	ND	4.5	86–90	73–78
3 (9)	Small gully of water cut through planting site; light at site good	ND	ND	ND	4 6[a]	ND	74–77
4 (11)	Apparently with good micro-conditions for the plant	320–350	769–800	18.5	4.4	86–90	73–78
5 (7)	Site with good light and moisture, wet below site	200–250	750–780	19.9	4.7	86–90	73–78
6 (7)	Good light and moisture	112–115	190–200	ND	4.4	86–90	73–78
7 (7)	Site with most light, driest of sites	ND	ND	9.6	ND	ND	ND
New population		ND	ND	16.8	4.8	ND	ND

[a] pH measurement taken of water flowing through this site rather than of soil.
[b] ND = measurement was not determined for that site.

Of the seeds germinated in these experiments, 13 from seed lot 942375 were established as propagating culture lines. These cultures multiplied slowly on the propagating medium, with one shoot giving rise to 2–5 shoots within eight weeks. Shoots produced roots on the propagating medium, as well as on WP medium with or without 0.5 mg/L IBA (Figure 2A). Roots formed on WP medium with IBA were shorter with fewer visible root hairs than roots formed in the absence of IBA. Rooted plants were successfully established in sandy soil (Figure 2B) and did well in very shady, humid conditions.

Cryopreservation

Shoot tips prepared by encapsulation vitrification showed good survival through all the steps of the procedure (Figure 3). Although there was some decrease in survival during the final step of LN exposure, survival through freezing averaged 71%, with a range of 62–94% depending on the trial, which was not statistically different from survival through the preparative steps of the procedure: preculture

on medium containing ABA and mannitol, encapsulation in alginate beads, and exposure to the PVS2 cryoprotectant. Six genetic lines of *M. cumberlandensis* have been banked in CREW's CryoBioBank. Samples of three lines from four banking trials that were removed from LN storage after 9–10.5 years showed survival rates of 0%–80%, depending on the line and vial. Survival appeared to be related to the time in LN storage and also with the number of microthaw events calculated for each sample (Table 3).

Experimental Outplanting

Among the plants in the experimental outplanting, there was a steady decline in the original plants through 6 years of monitoring, at which point, 12 of the original 63 plants were observed. Seedlings were first observed during the second summer (2007), and flowering plants and seedlings have been observed in every year following (Figure 4). Thus, as numbers of the original plants decreased, the appearance of new plants has caused the numbers in the experimental

Table 2.   Germination of seeds of *M. cumberlandensis*.

Seed lot	Number of seeds	Germination temperature*	Number germinated (days)[a]	Percent germination
911449	20	26°C	2 (11)	10%
	63	26°C	9 (8)	14%
942375	24	26°C	12 (6)	50%
	23	15°C/30°C	15 (23)	65%
	25	4°C	19 (78)	76%

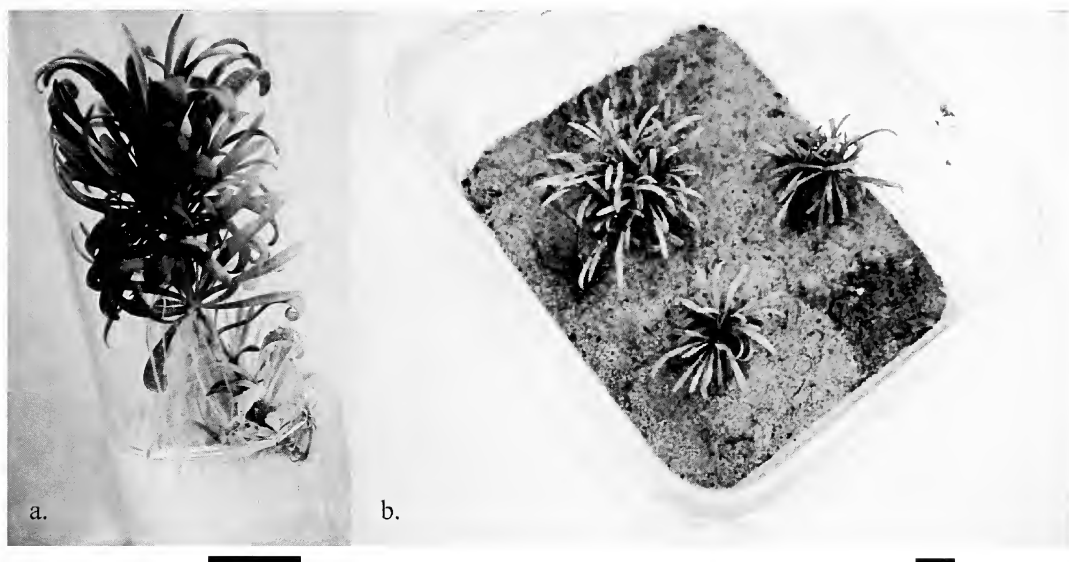[a] In each case, no further germination was observed beyond the time indicated.

Figure 2.   (A). *M. cumberlandensis* shoot with roots in vitro. (B) Acclimatized plants of *M. cumberlandensis.* (bars = 1 cm).
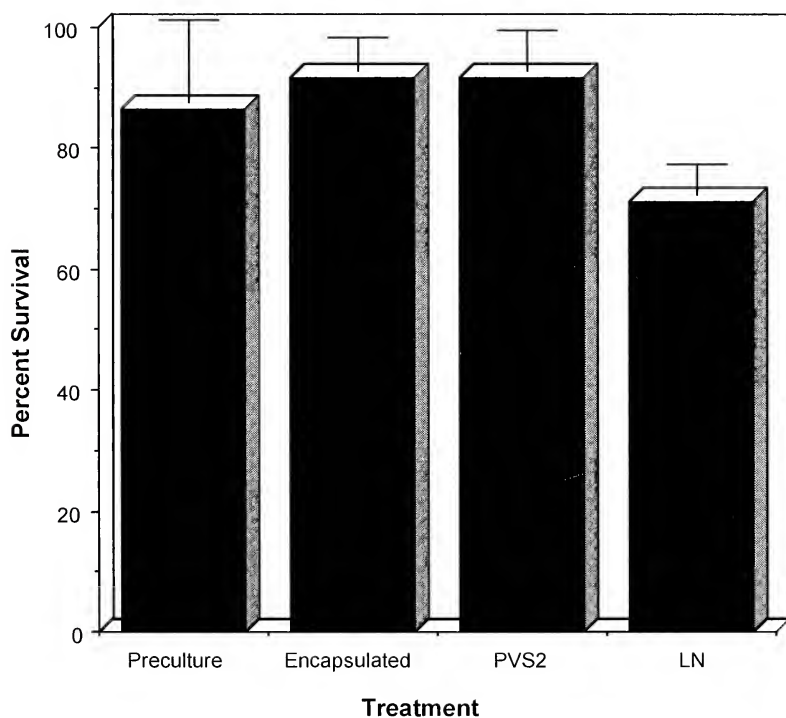

Figure 3.   Survival of *M. cumberlandensis* through the steps of cryopreservation using a modified encapsulation vitrification procedure (5): Preculture on 10 micromolar ABA and 0.3 M mannitol; encapsulation in alginate beads; PVS2 = 2 h in PVS2 cryoprotectant solution; LN = freezing in liquid nitrogen. Data represent average of four replicate trials. Error bars = standard errors; differences were not significant ($P > 0.05$, Tukey-Kremer).

Table 3. Survival of shoot tips of *M. cumberlandensis* after long-term liquid nitrogen storage.

Line	Years in LN storage	Number of "microthaw" events	Initial % survival	% Survival (Survival per vial)	% of Initial survival
AC - 6	9	10	87	75 (70, 80)	86
AC - 10	9.5	23	64	35 (20, 50)	54
AC - 1	10.5	66	63	15[a]	23
AC - 6	10.5	66	69	10 (0, 20)	14

[a] Samples from the two vials were mixed.

population to increase until they overtook the numbers of the original outplanting after five years (Figure 5). After this point, it became difficult to distinguish original plants from offspring, and survival of original lines could not be tracked further.

There did not appear to be any preferential survival due to genetic line (Figure 6), but there were differences in survival and multiplication based on site within the rockhouse (Figure 7). After six years, plants remained in four of the seven sites, disappearing from sites 3, 6, and 7 after 2 mo, 3 y, and 5 y, respectively. In contrast, four sites had plants after six years, including site 4 that had undergone explosive growth. All of the sites where measurements were taken had similar relative humidity, temperature, and soil pH; however, there appeared to be differences in light and soil moisture levels (Table 1). The best survival was observed in sites that had appeared to have moderate moisture and moderate light levels. Site 3 with water flowing through it and Site 7 that appeared to be the driest site, did not support survival.

Figure 4. Flowering plants and seedlings of *M. cumberlandensis* at the experimental outplanting site.

Best survival was seen in Sites 4 and 5, which had higher light and soil moisture levels than the other sites where these measurements were taken. Site 6 was originally described as having good moisture and light, but light levels measured here were lower than Sites 4 and 5, and this may have contributed to the decline of this group of plants. A new site was formed whereby14 plants were added in 2009 and 2010. In 2011, 19 plants were counted in the new site, indicating that this group was reproducing, as well.

DISCUSSION

To our knowledge, this is the first report of in vitro propagation of a species of *Minuartia* (*Arenaria*); however, there has been a large body of work on the culture of *Dianthus caryophylla* (6,7), as well as other species in the Caryophyllaceae, including *Pseudostellaria heterophylla* (8), *Gypsophila paniculata* (9), and *Lychnis senno* (10). Hyperhydricity has often been reported in cultures of *D. caryophylla* (11), but this has not been observed in *M. cumberlandensis*, possibly because the latter is native to a moist, humid environment. Because of this, it may be more adapted to the moist environment of tissue culture than *D. caryophylla* that is native to the Mediterranean climate and prefers well-drained soils and full sun.

Similarly, this appears to be the first report of shoot tip cryopreservation in *Minuartia*, although similar survival has been reported for *D. caryophylla*, also using the encapsulation vitrification procedure (12). The four samples of *M. cumberlandensis* removed from cryostorage after 9–10.5 years in LN showed varying degrees of survival. These survival rates were correlated with the time in storage, with the sample from 9 years of storage having the highest survival rates, close to the initial survival, and those from 10.5 years of storage having very low survival. The sample size
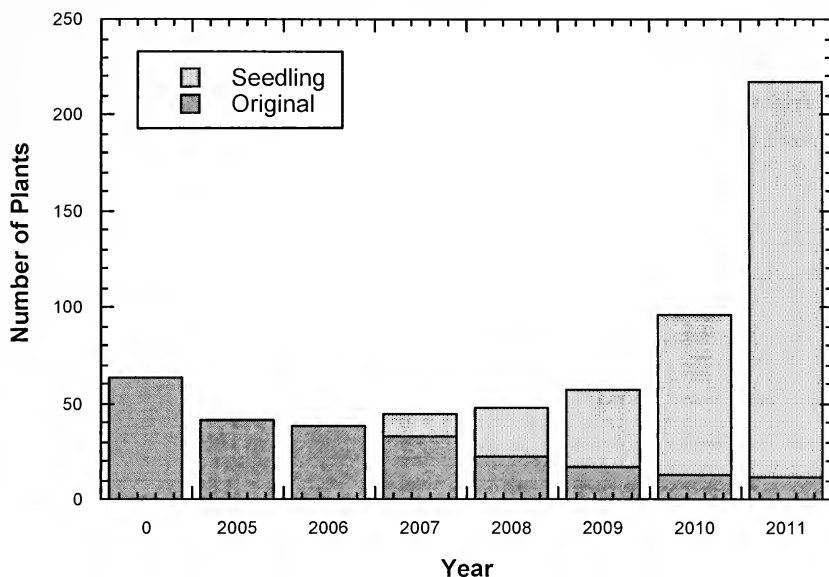
Figure 5. Survival of original plants and progeny at the experimental outplanting site during six years following planting (2005 = two months following planting).

tested here was small, and more samples need to be removed from storage to identify more clearly the cause of this loss in viability; however, if real, one explanation for this drop within 1.5 years could be that the time between 9 and 10 years of storage is critical for tissues of this species under these conditions and that the samples show a precipitous decline in viability during this time period. Alternatively, since the two older samples were banked on the same day, it is possible that some factor in the banking process led to their decline over the long-term, even though their initial survival was high. A third possibility is that the cumulative effect of "microthaw" events may have had an effect on
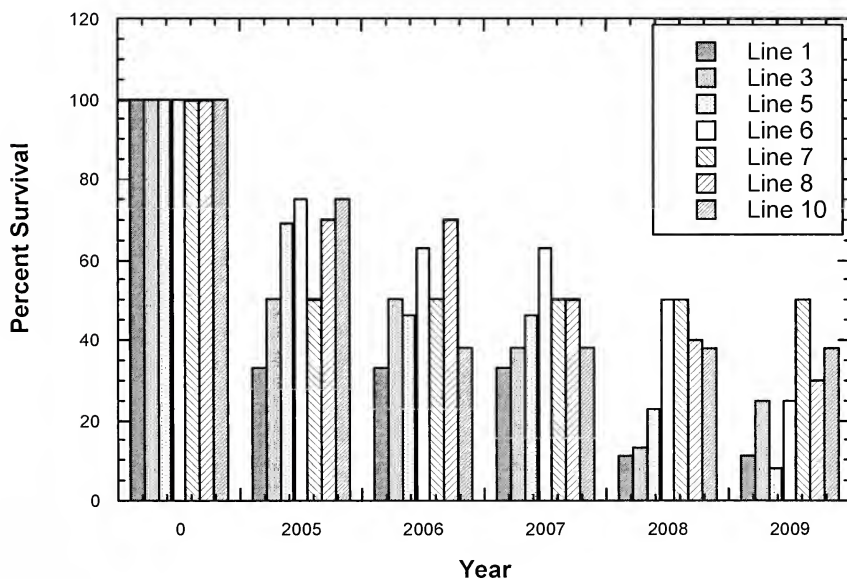

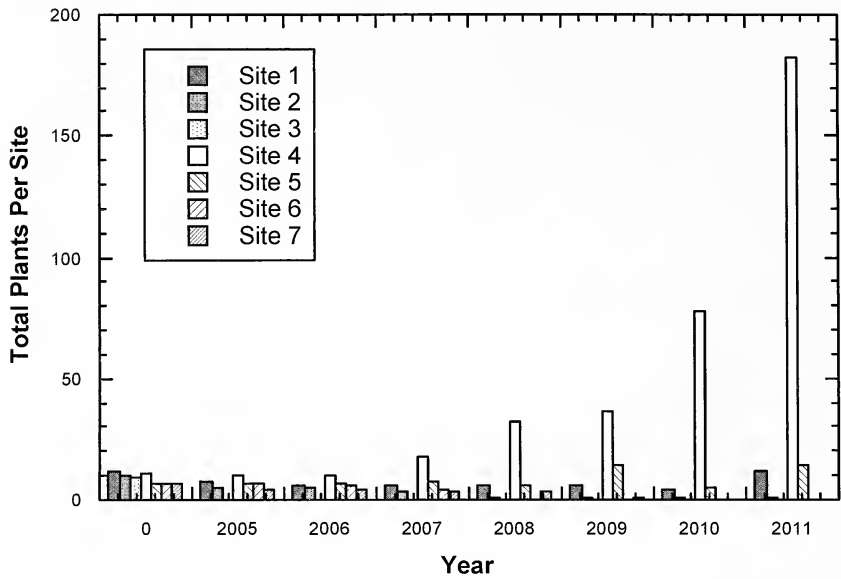Figure 6. Survival of original plants by genetic line from planting through 2009.

Figure 7.   Survival and growth of plants (original and progeny) in each site during the six years following planting.

the longevity of samples exposed to these short changes in temperature. If so, this may be an important consideration in future planning strategies for long-term tissue storage and the design of storage systems.

The in vitro propagated plants were successful in establishing a growing experimental population within the site of the rockhouse. Even though the original plant numbers continue to decline, the total number of plants continues to increase through the production of seedlings. The normal lifespan of this species is not known, but it is possible that the decline of the original plants is reflective of the natural history of this species. Future work will include genetic analysis of progeny, to evaluate the amount of genetic diversity of the growing population, in comparison with the founder plants.

The results of the experimental outplanting indicate that even within the relatively small area of a sandstone rockhouse, differences in microhabitat appear to be important in the survival and growth of outplanted *M. cumberlandensis*. This highlights the advantage of using tissue culture- or other clonally-propagated materials to test for appropriate habitat for outplanting rare species. It was possible to use several different genetic lines of *M. cumberlandensis* for the outplanting, but each line represented a clone of material that was

still maintained in culture in the laboratory. Thus, when plants were lost during the experimental outplanting, the genotype was not lost. Such an approach could be valuable when testing new habitat for rare species, as when attempting to establish new populations or in assisted migration in response to climate change (13).

The results of these studies demonstrate the feasibility of using in vitro methods to assist in addressing goals outlined in the recovery plan for *M. cumberlandensis* (2). One such goal is to "determine and implement the management necessary for long-term reproduction, establishment, maintenance, and vigor." Monitoring the in vitro-propagated plants in the experimental outplanting described here will contribute to the knowledge of how to establish these plants in the wild. Cryopreservation of established tissue lines will also contribute to another stated goal, to "maintain a cultivated source of plants." In addition, these methods contribute to Target 8 of the Global Strategy for Plant Conservation for ex situ conservation and restoration (14).

A further goal is to, "develop techniques and reestablish populations in suitable habitat within the species' historic range, if necessary, to recover the species." It is yet to be determined whether reintroduction projects

will be needed for the survival of this species; however, the results described here demonstrate that the techniques of in vitro propagation and outplanting are workable for *M. cumberlandensis* and that they could contribute to the overall efforts to conserve this species into the future.

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Watching and Listening to the Coefficient of Restitution

Marco Ciocca[1] and Jing Wang

Eastern Kentucky University, Richmond, KY

ABSTRACT

We discuss the measurement of the coefficient of restitution (COR) and the acceleration of gravity g using video and sound analysis with both Tracker and Logger Pro 3 software. Measuring COR of a bouncing ball is a common activity in physics labs from high school to intro level college courses (e.g., measuring COR to obtain gravitational acceleration). A typical experiment involves measuring the heights of subsequent ball bounces, although its precision is sometimes of concern. An indirect measurement by analyzing the sound of collisions is also popular and provides higher precision. In our experiment, video and sound analyses are integrated to provide both direct and indirect measurements of COR for pedagogical purposes. COR values by video analysis produce more accurate determinations of the gravitational acceleration compared with the COR from sound analysis alone.

KEY WORDS: Coefficient of restitution, video analysis, sound analysis, projectile motion

The COR between a ball and a surface is defined as the ratio of the ball's speeds after and before a collision. When a ball is dropped from an arbitrary height onto a surface, it will bounce several times before coming to rest. Further, neglecting air resistance, conservation of mechanical energy dictates that each rebound height depends on the vertical velocity after the previous collision, and determines the vertical velocity before the next collision. The COR can then be found by measuring the heights of sequential ball bounces.

An alternative method of "hearing" the COR became possible when computers came into introductory physics labs. (Bernstein 1977; Smith et al. 1981) Instead of measuring the height, the time interval between two subsequent bounces is measured from the digitized sound file recorded during the ball bounces. The COR is calculated as the ratio of the two consecutive time intervals. The sound analysis avoids issues related to the measurement of height, and provides higher precision than the traditional method. (Bernstein 1978) It also provides more accurate timing information than stopwatches. (9)

An important benefit of using the measurement of time intervals to obtain the COR is the determination of the gravitational acceleration g. (Guercio and Zanetti 1987) Given that the initial height of the ball can be readily measured, the time-of-flight between release moment and the first collision with the surface only depends on the gravitational

acceleration (neglecting air resistance). We can use COR to estimate the initial time-of-flight, hence determine g directly. However, there are two major issues associated with this method. First, COR sometimes depends on the speed of the ball. In such a situation, the estimation of the initial time-of-flight will not be simple (Bernstein 1977; Aguiar and Laudares 2003). Second, sound analysis just to obtain g is somewhat difficult for introductory physics labs. Ideally, the times of the bounces can be identified by marking the pulses in the sound file. In reality, the original sound file contains white noise and is very hard to analyze without filtering (e.g., using a running average filter or Laplace filter.) (Stensgaard and Laegsgaard 2001) Complicated preprocessing must be introduced in order to produce reliable data. Given its complexity, using sound analysis just to obtain g is rarely considered in introductory physics courses.

EXPERIMENT

Studies have shown that students who learn physics through video analysis projects show better data interpretation skills and deeper understanding on certain topics. (Beichner 1996; Laws and Pfister 1998) We designed therefore an experiment to help introductory physics students to measure the coefficient of restitution and the gravitational acceleration. Both the video and the sound of a bouncing ball are recorded. We used video analysis to measure the heights of the ball bounces and time-of-flights; we used sound analysis to check for consistency of the time-of-flights measurements. This approach, especially the use of

[1]To whom correspondence should be addressed. E-mail: Marco.Ciocca@eku.edu
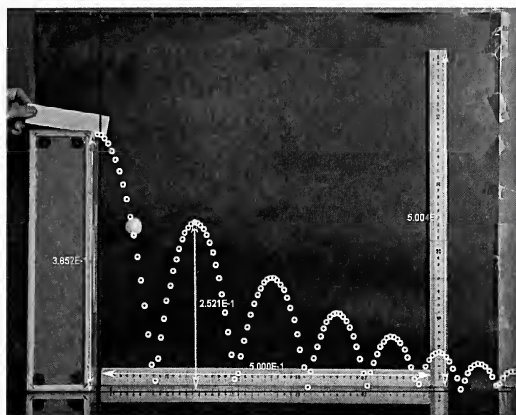
Figure 1. Video analysis of the ball bouncing process.

video, can prove very useful. For example, students often have difficulty grasping that in projectile motion there is no acceleration in the horizontal direction. The data collected in this experiment clearly show this concept. Video analysis is applied throughout the process, while the more complex sound analysis, a well-established method (Bernstein 1997; Smith and Jones 1981; Bernstein 1978; Aguiar and Pereira 2011; Guercio and Zanetti 1987; Aguiar and Laudares 2003; Stensgaard and Laegsgaard 2001) is used as an auxiliary method to demonstrate the similarity of the results that can be obtained.

DESIGN

2A hard plastic ball was chosen, small and smooth to reduce air friction to a negligible level, and of high elasticity so that a large number of bounces could be observed. To perform measurements on the trajectory of the ball, we recorded several videos of this ball falling on a hard black acrylic surface (see Figure 1) and the data presented here were obtained by analyzing one of them. We conducted the analysis using the motion-tracking freeware Tracker (version 4.5) (Brown and Christian 2011). This particular software allows the user to analyze a video frame-by-frame. The video file characteristics (resolution and time differential between frames) are automatically determined by the program. To complete the frame analysis, it is necessary to establish a starting point and a reference scale in the video frames. This needs to be done just once. Following this determination, Tracker

will allow to determine positions and compute speeds of the object under study. We set the scale by having a horizontal ruler (L = 0.500 m) present in all the frames analyzed.

Parallax issues could distort the image and cause the horizontal scale to be different from the vertical scale. To solve this problem, we carefully placed the camera with respect of the ball's path so that the camera's focal plane was parallel to the plane of the ball's trajectory. To ensure the video has the same scale for both vertical and horizontal coordinates we added a vertical ruler (of known length L = 0.500 m) within the frame (see Figure 1). Using the scale set by the horizontal ruler, the length of the vertical ruler on the video was measured to be exactly 0.500 m.

The position of the ball in flight (including its release point and its rebound heights) are determined by simply "clicking" with a crosshair on the image of the ball in flight. Due to the 1/2000 s shutter duration (see more discussions in section 4), the image of the ball is effectively "freeze-framed." By using the bottom of the ball as the reference point, we can reduce the uncertainty of its position to one pixel. Since the vertical resolution of the frame is 720 pixels, and that the height of the frame was about 0.6 m, this corresponds to an uncertainty of approximately 0.001 m.

In Tracker software, each individual video frame has a time-stamp associated with it, thus allowing determination of the time elapsed between successive ball positions. Due to the frame rate of the video, 59.95 frames per second (fps), the shortest time interval is 0.0167 s, the reciprocal of fps.

MEASURING COEFFICIENT OF RESTITUTION

Here we measure the COR from two different approaches: video analysis (using Tracker 4.5), and sound analysis (using Logger Pro 3). In video analysis, we measure the initial height of the dropped ball, and then the heights of several subsequent bounces. As shown in Figure 1, all the heights can be directly measured from the video. Neglecting air resistance, from conservation of mechanical energy each rebound height is proportional to the square of the vertical velocity after the collision. The COR e of the nth hit is then determined as

Table 1. Coefficient of restitution measured from video and sound.

| Collision No. | Video Analysis | | Sound Analysis | |
	Height (m) (±0.001 m)	COR of the nth collision	Time-of-flight between the nth and the $(n + 1)^{th}$ collision (s) (±0.0003 s)	COR of the nth collision
initial	0.385	N/A	N/A	N/A
1	0.252	0.808 ± 0.004	0.4440	N/A
2	0.170	0.821 ± 0.006	0.3618	0.815 ± 0.002
3	0.117	0.830 ± 0.009	0.2979	0.823 ± 0.002
4	0.082	0.835 ± 0.013	0.2475	0.831 ± 0.003
5	0.059	0.846 ± 0.019	0.2058	0.832 ± 0.004
6	0.041	0.838 ± 0.027	0.1734	0.843 ± 0.004

$$e_n = \sqrt{\frac{h_n}{h_{n-1}}}, \qquad (1)$$

where h_n is the rebound height, and h_{n-1} is the previous height. The CORs calculated from video analysis are listed in Table 1 and show that the COR is not a constant. The COR and its uncertainty tend to increase as the velocity (or height) decreases. The COR measured from the first collision has the least uncertainty.

The COR of the ball-surface collision was also measured using sound analysis, with a technique similar to the that of Aguiar et al. (2003). The sound of the ball hitting the surface was recorded using a Vernier microphone connected with a LabPro interface and collected with Logger Pro 3 (version 3.8.2). (Vernier 2010) The microphone was placed within a meter of the ball initial position. The sound can be digitized either by recording the collisions as they happen or by re-recording the audio captured by the camcorder at a later time. Both methods yielded the same results which suggests the placement of the microphone is not critical. In our experiment, the sound is digitized with a 3000 Hz sampling rate, determined by the amount of memory available in the interface. The COR of the nth bounce is then obtained by the ratio of two sequential bounces' time-of-flights as following:

$$e_n = T_n / T_{n-1}. \qquad (2)$$

Using the method above, the sound file recorded is clean enough so that preprocessing was not necessary. Typical sound pressure variation caused by a collision is plotted in Figure 2. In this raw data plot, the white noise is smoother while the collision sound generates an abrupt variation. This abrupt change clearly indicates the occurrence of collision at the first sharp peak.

The CORs measured from sound analysis are also listed in Table 1. Since the time interval between the release and the first collision is difficult to measure (it would require a triggering mechanism further complicating the experiment), from sound analysis we can only find the COR starting from the second bounce. The COR measured from the video and the sound are in good agreement. The uncertainty of the COR in sound analysis, unlike in video analysis, only increases slightly as the number of collision increases. In video analysis, the ball bounce height decreases to one tenth of the original height after six bounces. In sound analysis, however, the time of flight is only reduced to a third of its original value after six bounces, while the absolute uncertainty of both remains the same. ($\Delta h = \pm 0.001$ m, $\Delta t = \pm 0.0003$ s.) Hence the error of COR increases as the bounce number increases. This also results in a larger increase of the error in video analysis.

MEASURING G FROM VIDEO AND SOUND

The measurement of COR allowed us to calculate the gravitational acceleration g precisely. Given the initial height of the ball h_0 and the time-of-flight between the release moment and the first collision t_0 (neglecting air resistance), g is determined by the following equation:

$$g = 2h_0 / t_0^2. \qquad (3)$$

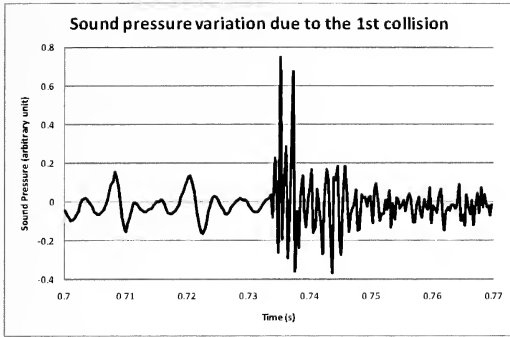The initial height can be found by measuring the height of the release platform. However, it

Figure 2.   Sound pressure variation due to the 1st ball-surface collision.

is difficult to measure t_0. We will need the COR measured from the previous step. If the COR at the 1st collision is known, we obtain

$$t_o = \frac{1}{2}\left(\frac{t_1}{e_1}\right), \tag{4}$$

where e_1 is the COR at the first 1st collision, and t_1 is the time-of-flight between the 1st and 2nd collisions.

In video analysis, e_1 is measured directly, and t_1 can be found from the position vs. time data. However, in sound analysis e_1 is not directly measurable. Since COR is not a constant over the range of bounces studied (Figure 3), we can use the fit in Figure 2 to compute e_1. The calculated g from video and from sound are both listed in Table 2.

Instead of calculating g using COR, we can also obtain g from the velocity data given by video analysis alone. From the ball position as a function of time, Tracker calculates the velocity of the ball. Figure 4 shows the results of a linear fitting to the vertical velocity. The slope of the v_y vs. t will give the acceleration of gravity g. The
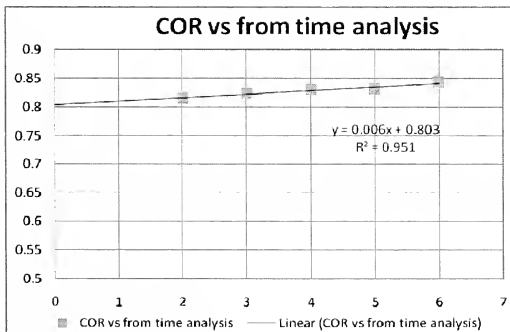
Table 2.   Gravitational acceleration measured from video and sound.

Variables	Video Analysis	Sound Analysis
h_0	0.385 m $\pm$ 0.001 m	0.385 m $\pm$ 0.001 m
e_1	0.808 $\pm$ 0.004	0.810 $\pm$ 0.004
t_1	0.450 s $\pm$ 0.001 s	0.444 s $\pm$ 0.0006 s
t_0	0.279 s $\pm$ 0.003 s	0.274 s $\pm$ 0.003 s
g	9.91 m/s^2 $\pm$ 0.08 m/s^2	10.2 m/s^2 $\pm$ 0.10 m/s^2

fitted data corresponds to the first four complete bounces. The agreement is excellent. The statistical error increases if the number of data points decreases; hence, we limited the fit to the first 4 bounces. The g values obtained from Figure 4 are listed in Table 3.

Video analysis therefore allows the experimenter to obtain measurement of the COR that are consistent with the more complicated sound analysis, and further permits determination of the acceleration of gravity g in a much more immediate way. Another benefit is that the projectile motion (between bounces the ball is indeed in free fall with a horizontal velocity component) can be used to show that acceleration in the horizontal direction is absent. This is often a difficult point for students of introductory physics. As shown in Figures 5, the ball's horizontal position as a function of time clearly points to uniform motion, as expected.

EQUIPMENT OPTIONS

The videos analyzed in this paper were captured by a SONY PMW-EX1 High Definition Digital Camcorder. The edited files have a stated resolution of 880 $\times$ 720 pixels at a nominal 60 fps (59.95 fps as measured with Tracker). By adopting a shutter speed of
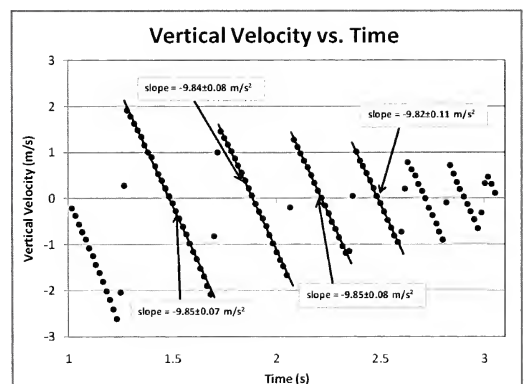

Figure 3.   COR vs. bounces from sound analysis.

Figure 4.   Vertical velocity vs. time graph.

Table 3.   Gravitational acceleration obtained from the vertical velocity data.

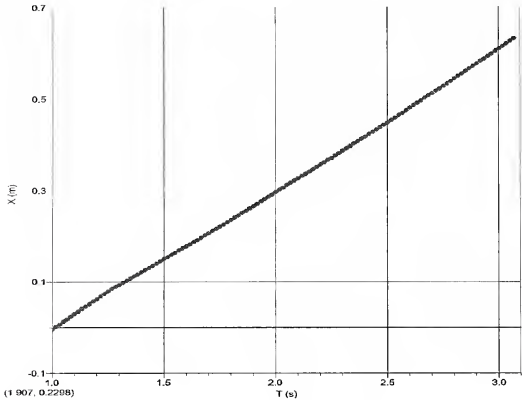| Bounces	g (m/s²)
1	9.85 ± 0.07
2	9.84 ± 0.08
3	9.85 ± 0.08
4	9.82 ± 0.11

Figure 5.   The ball's horizontal position vs. time graph.

1/2000 s the flight path of the ball was recorded with little blurring.

To achieve the best results in video analysis, one of the important features of the camcorder used is the ability of manually setting the shutter speed. Without manual control, usually the firmware of cameras maximizes exposure especially in low-light situations. Videos so obtained will have low noise, but objects moving rapidly will appear blurry due to the long time the shutter is open. To capture fast objects, the shutter opening time needs to be short. With manual control, a certain shutter speed can be selected, but the gain (or ISO settings, if using old style controls) of the camera needs to be increased. The video will become noisier, but the motion of the object will be much better defined.

As mentioned previously we used a semi-professional camcorder capable of a very short shutter time (1/2000 s) and a large frame rate (60 fps). Comparable data can be obtained by a less-expensive camcorder, provided that it possesses a manual setting. We have in fact used a Panasonic PV-Gs150 (purchased in 2006) with a 30 fps frame rate and a 1/250 shutter speed. Admittedly, the data are coarser (30 fps vs. 60 fps) but the results are in good agreement with the data obtained in

the previous section using the high definition camcorder. The dynamical measurements of g, from the slope of v_y vs. t still yield very good values of g, albeit with a larger statistical error. We can also analyze the data by measuring the heights of the bounces, from which COR are obtained in good agreement with the previous data, but with larger uncertainties as well.

SUMMARY

With video analysis and sound analysis integrated, we were able to match the results of sound analysis in obtaining COR values. The method also offers a more reliable determination of the acceleration of gravity g and offers a better pedagogical understanding of the concepts relating to projectile motion.

ACKNOWLEDGEMENT

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Quantitative Proteomic Analysis of Differentially Expressed Proteins in Aβ(17-42) Treated Synaptosomes

Jaffer Mohammed, Moses Henderson, Rebecca Williams, and Tanea T. Reed[1]

Department of Chemistry, Eastern Kentucky University, Richmond, Kentucky 40475-3102

and

Rukhsana Sultana and Joshua Owen

Department of Chemistry, University of Kentucky, Lexington, Kentucky 40506-0055

ABSTRACT

Oxidative stress is associated in the pathogenesis of numerous diseases such as various neurodegenerative disorders, ischemia, and cancer. The brain is susceptible to oxidative stress due to its high level of polyunsaturated fatty acids, increased rate of oxygen consumption, and elevated levels of free radicals. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) can react with biomolecules such as proteins, lipids, carbohydrates, and nucleic acids, which leads to oxidative damage, cellular dysfunction, and ultimately cell death. Down syndrome (DS), a genetic abnormality, is the most common form of mild to moderate mental retardation in newborn infants. DS patients have extensive deposition of Aβ(17-42) peptide that could contribute to their increased rate of developing Alzheimer's disease (AD), which is consistent with current research. Because AD cannot be properly diagnosed until autopsy, development of a novel Down syndrome model using Aβ(17-42) could be beneficial in determining oxidative stress levels and their relationship to mild cognitive impairment (MCI), the earliest form of AD. This work will demonstrate the use of a novel Down Syndrome model and its correlation to oxidative stress. We have found a significant difference between oxidative stress levels in Aβ(17-42) treated synaptosomes and control synaptosomes. Using proteomics, we also identified several biomarkers including aldehyde dehydrogenase, aldolase, α-enolase, heat shock cognate 71, peptidyl-prolyl cis-trans isomerase, and ATP synthase α chain. Our present findings suggest the role of Aβ(17-42) as one of the contributing factors in mediating oxidative stress in the DS and AD brain that can lead to neurodegeneration. This novel DS model may have potential applications as a diagnostic tool to identify biomarkers that may contribute to AD.

KEY WORDS: oxidative stress, Down syndrome, Alzheimer's disease, amyloid beta peptide, neurodegeneration

Oxidative stress is associated in the pathogenesis of numerous diseases such as neurodegenerative disorders, ischemia, and cancer. Under oxidative stress conditions, the balance between the prooxidant and antioxidant species is impaired and, as a result, ROS and RNS are produced. ROS and RNS have the ability to react with biomolecules including carbohydrates, proteins, lipids, and nucleic acids that leads to oxidative damage and ultimately cellular dysfunction (29). The brain is susceptible to oxidative stress due to its high level of polyunsaturated fatty acids, increased rate of oxygen consumption, elevated levels of free radicals, and comparatively low levels of antioxidant defense systems (22). The typical markers of oxidative stress commonly studied to determine the oxidative stress levels include protein carbonyls, 3-nitrotyrosine (3-NT), free fatty acid release, 4-hydroxy-2-nonenal (HNE), acrolein, advanced glycation end products for carbohydrates, iso-and neuroprostane formation, 8-OH-2'-deoxyguanosine, and altered DNA repair mechanisms (29).

Amyloid beta (Aβ), a 40–42 amino acid peptide is formed by the proteolytic cleavage of amyloid precursor protein (APP) via β and γ secretases. It has been discovered that mutations in APP, presenilin-1, or presenilin-2 lead to increased production of Aβ(1–42) and the early onset of AD (29). If APP is cleaved by α- and γ-secretases, however, the Aβ(17-42) fragment, also known as the p3 fragment, is formed (Figure 1).

DS is the most common genetic cause of mild to moderate mental retardation occurring

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$^+_3$HN-LVFFAEDVGSNKGAIIGLMVGGVVIA-COO$^-$

Figure 1.   Primary structure for Aβ(17–42).

in newborn infants. DS is characterized by a trisomy at chromosome 21, the location of APP. DS persons also demonstrate elevated levels of oxidative stress and Aβ(17-42) deposition (17). They undergo premature aging and develop Alzheimer's-like brain changes during their 30 s or 40 s leading to dementia throughout their life. DS persons have a higher risk for AD and develop this debilitating disorder at a younger age compared to typical AD onset.

Protein expression analysis can potentially aid in better understanding of pathways involved in pathogenesis of AD; therefore, depicting the mechanistic progression of AD (28). Protein oxidation can lead to loss of protein function, abnormal protein turnover, imbalance of cellular redox potential, interference with cell cycle, and eventual cell death, all of which are observed in AD (2). Previous literature demonstrates that the proteins oxidized in the DS and AD brain affect various cellular functions including energy metabolism, proteosome function, glutamate uptake and excitotoxicity, neuritic connections, and neuronal communication (2,17).

Proteomics can be defined as the systematic study of proteins produced in a single cell or organism. The field of proteomics is used to provide the complete view of the structure, function, and regulation of a given cell, tissue, or organism. Protein expression is found to be altered in disease conditions; hence, proteomic studies can serve as a sensitive technique to expand insight into a host of biologic processes and phenotypes of diseased and normal cells (28). Because there is an underlying correlation between DS and AD, and definitive AD diagnosis can only be conducted at autopsy, the need for an AD diagnostic system is great. The goal of this work is to develop a novel model of DS using Aβ(17-42) peptide that may have potential applications as a diagnostic tool to identify biomarkers that may contribute to AD.

MATERIALS AND METHODS

All chemicals were of the highest purity and obtained from Sigma Aldrich (St. Louis, MO). Aβ(17-42) peptide was obtained from Ana-

Spec (San Jose, CA) with HPLC and MS purity verification. The peptide was stored at −20°C until used. The OxyBlot protein oxidation detection kit was obtained from Chemicon International (Temecula, CA). SY-PRO Ruby stain was obtained from Bio-Rad (Hercules, CA).

Synaptosomal Preparation

Synaptosomes were isolated from the brain tissue of Mongolian gerbils. A total of 12 Mongolian gerbils (six control and six experimental) were used in this experiment. Amyloid beta peptide Aβ(17-42) (AnaSpec, San Jose, CA) was dissolved in phosphate buffered saline (PBS) to a final concentration of 0.5 mg/mL and preincubated at 37°C for 24 h prior to incubation with synaptosomes. The experimental set of synaptosomal preparations was incubated with Aβ(17-42) for 6 h at 37°C to induce oxidative stress. The animals were fed standard Purina rodent laboratory chow and housed in the University of Kentucky's Central Animal Facility in a 12 h light/dark phase. Synaptosomes were isolated from three-month old Mongolian gerbils at the University of Kentucky in their Division of Laboratory Animal Research Center. The animals were briefly anesthetized and sacrificed. Their brains were immediately isolated and dissected following sacrifice. The brain was homogenized with a tissue homogenizer, placed in ice containing 0.32 M sucrose isolation buffer [leupeptin (4 μg/mL), pepstatin (5 μg/mL), aprotinin (5 μg/mL), 2 mM ethylene glycol-bistetraacetic acid, 2 mM ethylenediaminetetraacetic acid, 20 mM 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid, trypsin inhibitor (20 μg/mL), and 0.2 mM phenylmethanesulfonyl fluoride, pH 7.4]. The homogenate obtained was centrifuged at 1500 g for 10 minutes. The supernatant was then centrifuged at 20,000 g for 10 minutes. The pellet obtained was resuspended in 0.32 M sucrose isolation buffer. Sucrose solutions (0.85 M pH 8, 1.0 M pH 8, and 1.18 M pH 8.5) were prepared and layered in plastic centrifuge tubes to form a discontinuous sucrose gradient. The tubes were then centrifuged at 82,000 g for 60 min at 4°C. Synaptosomes were collected from the sucrose interface of 1.0 M/1.18 M layer and washed in Locke's buffer [154 mM sodium bicarbonate, 5 mM

glucose, 5 mM 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (HEPES), pH 7.4] twice for 10 min at 32,000 g. The control synaptosomes were synaptosomes that were not incubated with Aβ(17-42) peptide. The synaptosomes obtained were assayed to determine protein concentration by using Pierce bicinchoninic acid assay method (3).

Oxidative Stress Parameter Measurement

Measurement of protein carbonyls, protein nitration (3-nitrotyrosine), and lipid peroxidation (HNE) was performed in a similar manner for each parameter. Five microliters of brain homogenate was incubated with 5 µL of Laemmli buffer [0.125 M Tris base, pH 6.8, 4% (v/v) SDS, and 20% (v/v) glycerol] for 20 minutes. The resulting sample (250 ng) was loaded per well in the slot blot apparatus containing a nitrocellulose membrane under pressure. After the sample was loaded, the membrane was blocked with 3% (w/v) bovine serum albumin (BSA) in phosphate buffered saline containing 0.01% (w/v) sodium azide and 0.2% (v/v) Tween 20 (PBST) for 1 h and then incubated with a 1:5000 dilution of anti-HNE polyclonal antibody in PBST for 2 h. After primary antibody incubation, the membrane was carefully washed three times for five minutes. A secondary antibody of anti-rabbit IgG alkaline phosphatase in PBST (1:3000 dilution) was added to the membrane for 1 hr. The membrane was again washed three times in PBST and developed using Sigmafast BCIP/NBT (5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium) tablets (Sigma, St. Louis, MO). For determining levels of 3-nitrotyrosine (3-NT), the only notable difference is the dilution for the primary 3-NT antibody (1:2000). For measurement of protein carbonyls, a polyclonal 2, 4-dinitrophenylhydrazine antibody (Millipore, Billerica, MA) was used in a 1:1500 dilution. Blots were dried, scanned with Adobe Photoshop (San Jose, CA), and quantitated with Scion Image software.

Two Dimensional Gel Electrophoresis

To obtain a precipitate, 100% cold trichloroacetic acid (TCA) was added to 150 µg of sample to achieve a final concentration of 15% TCA, and then it was placed on ice for 10 minutes. The precipitates obtained were centrifuged at 4000× g for 2 min at 4°C. The resulting protein pellets were washed three times with 1 mL of 1:1 (v/v) ethanol: ethyl acetate solution. The protein samples were dissolved in 200 µL of rehydration buffer (8 M urea, 20 mM DTT, 2 M thiourea, 2.0% (w/v) CHAPS, 0.2% Biolytes and bromophenol blue). 200 µL of sample solution was added to each pH 3–10 IPG strip (Bio-Rad Hercules, CA), and constant voltage (50 V) was applied for one hour. Mineral oil was added to the top of each strip to prevent the evaporation. The IPG strips were then actively rehydrated on an IEF cell (Bio-Rad Hercules, CA) for 16 h at 50 V. Isoelectric focusing of proteins was performed at 20°C under the following conditions: 300 V for 2 h linearly, 500 V for 2 h linearly, 1000 V for 2 h linearly, 8000 V for 8 h linearly, and 8000 V for 10 h rapidly. The strips were then stored at −80°C until future use.

For SDS-PAGE, the gel strips obtained after isoelectric focusing were allowed to thaw. The thawed strips were then equilibrated for 10 min in equilibration buffer (50 mM Tris-HCl (pH 6.8) containing 6 M urea, 0.5% DTT, 1% (w/v) sodium dodecyl sulfate (SDS), 30% (v/v) glycerol). After 10 min, they were reequilibrated in the same buffer for 10 min; however, this time DTT was replaced by 4.5% iodoacetamide (IA). Control and Aβ(17-42) strips were placed on 8–16% precast Criterion Tris-HCl gels (Bio-Rad, Hercules, CA). Electrophoresis was performed for 65 min at 200 V in a 2D gel electrophoresis gel apparatus (Bio-Rad, Hercules, CA).

Staining and Image Analysis

Following electrophoresis, the gels were incubated with fixing solution [10% (v/v) methanol and 7% (v/v) acetic acid] for 20 min. The gels were then stained with fluorescent SYPRO Ruby gel stain (Bio-Rad, Hercules, CA) for 2 h on a platform rocker. After staining, the gels were placed in distilled water overnight.

After destaining, each 2D gel was placed under a UV transilluminator (EDVOTEK, Bethesda, MD) to visualize the protein spots. Gels were stored in distilled water at 4°C until spot excision for in-gel trypsin digestion. The images were analyzed for significant differences in protein spot intensity between the control and Aβ(17-42) treated synaptosomes

by using PDQuest 2-D image analysis software (Bio-Rad, Hercules, CA). After the images were analyzed and matched by the PDQuest software, the normalized intensity of each protein spot from individual gels was compared between groups for statistical analysis using Student t-test. P values of less than 0.05 were considered to be significant.

Trypsin Digestion

For mass spectrometric analysis, the statistically significant protein spots were excised from the gels by the method described by Thongboonkerd (30). These protein spots were excised from the 2D gels and transferred into new clean microcentrifuge tubes. The gel pieces were incubated with 0.1 M ammonium bicarbonate (NH_4HCO_3) for 15 min. To the same tube, acetonitrile was added to the gel pieces containing ammonium bicarbonate and incubated at room temperature for 15 min. Ammonium bicarbonate and acetonitrile solvents were removed after 15 min and gel pieces were allowed to dry in laminar flow hood for 30 min. The gel pieces were rehydrated with 20 mM DTT (Bio-Rad, Hercules, CA) prepared in 0.1 M NH_4HCO_3 and incubated at 56°C for 45 min. The DTT solution was removed and 55 mM iodoacetamide (Bio-Rad, Hercules, CA) prepared in 0.1 M NH_4HCO_3 was added to the gel pieces and incubated for 30 min in the dark at room temperature. The iodoacetamide solution was removed and replaced with 50 mM NH_4HCO_3 and incubated for 15 min at room temperature. To the same tube, acetonitrile was added and incubated at room temperature for 15 min. The mixture of ammonium bicarbonate and acetonitrile solutions were removed from the tube, and the gels were allowed to dry in a laminar flow hood for 30 min. Following drying in the laminar flow, the gel pieces were rehydrated with 20 ng/μL of modified trypsin (Promega, Madison, WI) in 50 mM NH_4HCO_3 with the minimal volume to cover the gel pieces and allowed to incubate overnight (~18 h) with shaking at 37°C. To prepare the protein digests for mass spectrometry analysis, the digests were attached to mini chromatography columns, Supelco Ziptips, (Millipore Corporation, Billerica, MA) via pipetting in four different buffers: (i) 5% acetonitrile, 0.1% formic acid,

(ii) 95% acetonitrile, 0.1% formic acid, (iii) 100% acetonitrile, and (iv) 50% acetonitrile, 0.1% formic acid. After multiple washings with the different buffers, the eluent was drawn and gently expelled several times to remove the sample from the Ziptip column completely. The tubes containing the peptides were sent to the University of Louisville's Core Mass Spectrometry Facility for mass spectrometric analysis and protein identification.

Mass Spectrometry and Database Searching

All mass spectra were recorded at the University of Louisville Core Mass Spectrometry Facility recorded on a LTQ Orbitrap XL mass spectrometer connected with a nanospray nanomate ionization source. Tryptic peptides were analyzed with an automated nanospray Nanomate Orbitrap XL MS/MS platform under the following conditions: injection time 50 ms, 35% collision energy, MS/MS spectra were measured in the FT at 7500 resolution, and dynamic exclusion was set for 120 s. MS/MS spectra were searched against the ipi_Rodent database using SEQUEST with the following criteria: Xcorr >1.5, 2.0, 2.5, 3.0 for +1, +2, +3, and +4 charge states, respectively, and P-value (protein and peptide) <0.01. IPI accession numbers were cross-correlated with SwissProt accession numbers for the final protein identification.

Enzymatic Assays

Alpha enolase is responsible for the conversion of 2-phosphoglycerate to phosphoenolpyruvate in glycolysis. The enzyme activity is measured spectrophotometrically at 340 nm. The reaction mixture (0.1 ml final volume) contained: 2 mM Na_2HPO_4 (pH 7.4), 400 mM EDTA, 2 mM 2-phosphoglycerate. The assay was carried out at 25°C. Aldolase is the glycolytic enzyme that cleaves fructose 1, 6-bisphosphate to glyceraldehyde-3-phosphate and dihydroxyacetone phosphate. During this reaction, β-nicotinamide adenine dinucleotide (β-NADH) is oxidized and its absorbance is measured at 340 nm. The assay was carried out at 25°C in a reaction mixture containing 58 mM fructose 1, 6-bisphosphate, 4 mM β-NADH, and 50 U glycerophosphate dehydrogenase/triose phosphate isomerase solution.
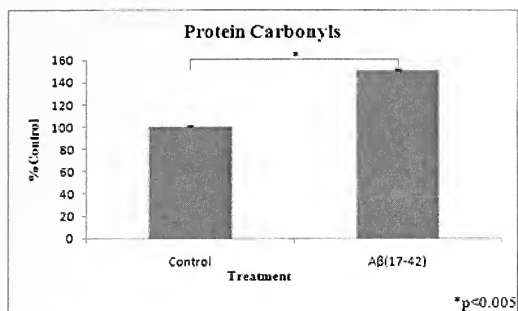
Figure 2. Levels of protein carbonyls in control synaptosomes compared to Aβ(17–42) treated synaptosomes. Error bars indicate the SEM for each group measured (n = 6).

Figure 4. Protein bound HNE levels in control synaptosomes compared to Aβ(17–42) treated synaptosomes. Error bars indicate the SEM for each group measured (n = 6).

RESULTS

Slot blot analysis was performed for control synaptosomes and Aβ(17-42) treated synaptosomes. Statistical analysis using Student's t-test was conducted and probability (p) values of less than 0.05 were considered to be significant. Based on the Student's t-test, levels of protein carbonyls (Figure 2), protein nitration (Figure 3), and HNE levels (Figure 4) were calculated and a significant difference was found between the control and the experimental set for each oxidative parameter. The level of protein carbonyls was elevated 45% compared to control synaptosomes; whereas 3-NT and HNE levels increased 10% and 15%, respectively. To assess whether there were any changes in the proteomic profile between the control and Aβ(17-42) treated synaptosomes, differential protein expression was measured by the differences in densitometric intensities on the gels. After determining the differentially expressed protein spots in PDQuest software,
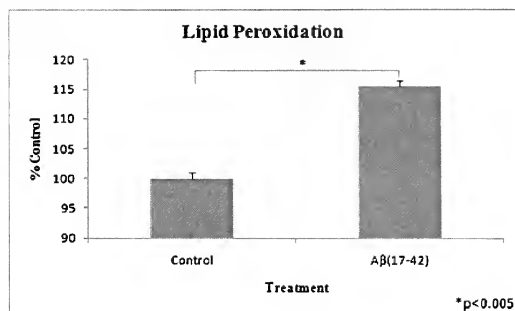
these spots were excised from the 2D gel and in-gel trypsin digestion was performed. Table 1 gives an overall summary of the proteins identified by mass spectrometry. Five proteins showed increased expression, whereas one protein showed decreased expression between the experimental and control set. Mass spectrometry analysis allowed for the identification of these differentially expressed proteins. The following enzymes were identified: aldehyde dehydrogenase, aldolase, ATP synthase, alpha

Figure 5. 2D gel map of synaptosomal proteins identified by mass spectrometry for Aβ(17–42) treated synaptosomes (*A*) and control synaptosomes (*B*). Proteins showing differential expression are shown as the circled spots.

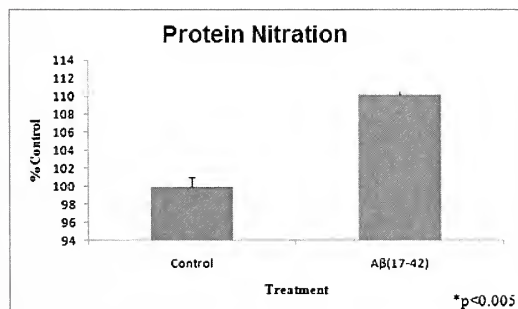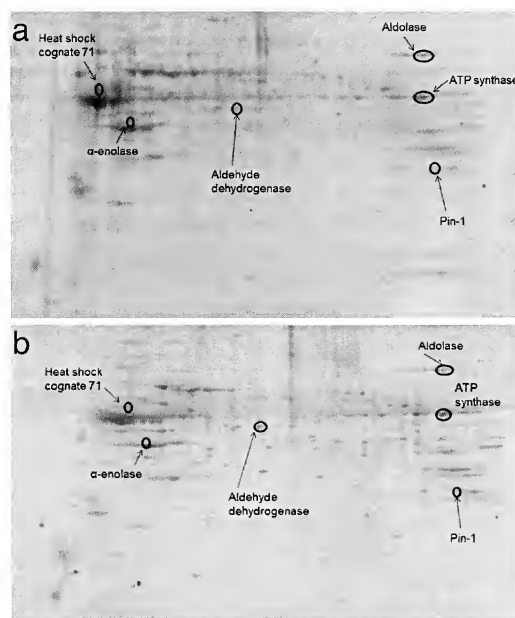Figure 3. Protein nitration levels of control synaptosomes versus Aβ(17–42) treated synaptosomes. Error bars indicate the SEM for each group measured (n = 6).

Table 1.   Expression profile of significantly differential expressed proteins.

Protein Identification	Peptide matches	pI	MW (kDa)	Protein expression	P value
Aldehyde dehydrogenase	2/3	7.53	56.5	Upregulated	<0.005
Aldolase	2/2	8.30	39.3	Upregulated	<0.005
Alpha (α) enolase	3/5	6.37	47.1	Upregulated	<0.0003
ATP synthase	3/4	9.22	59.7	Down regulated	<0.002
Heat shock cognate 71	10/17	5.37	70.8	Upregulated	<0.003
Peptidyl-prolyl cis-trans isomerase	2/2	5.38	64.7	Upregulated	<0.002

enolase, dihydrolipoyllysine acetyltransferase, peptidyl-propyl cis-trans isomerase (Pin-1), and heat shock protein 71 (Table 1).

Enzyme assays were carried out for alpha enolase and aldolase in control and Aβ(17-42) treated synaptosomes. Although a reduction in enzymatic activity was observed in both enzymes, it was deemed not significant ($P < 0.25$ for enolase (Figure 6) and $P < 0.1$ for aldolase (Figure 7)).

DISCUSSION

Synaptosomes are isolated terminals of neurons prepared by homogenization and fractionation of nerve tissue. First isolated by Hebb and Whittaker (11), they were later identified by electron microscopy as detached synapses (10). The amyloid beta peptide inserts itself into the lipid bilayer; therefore, synaptosomes are an excellent model to study the consequences of protein oxidation via peptide aggregation. We examined the specific oxidative stress effects of Aβ(17-42) on synaptosomes. As Aβ(17-42) peptide is found in DS and AD, it could contribute to the development of AD at an earlier age in DS patients. The oxidative stress induced by Aβ(17-42) suggests that it could be one of the contributing factors in the pathogenesis of AD. It has been shown that the Met35 residue Aβ(1–42) is a key amino acid residue involved in amyloid beta-peptide mediated toxicity and, consequently, the pathogenesis of AD (3). Parallels can be drawn that Met35 found in the Aβ(17-42) also could contribute to the oxidative stress induced by this peptide.

2D gel electrophoresis separates a mixture of proteins into single easily detectable protein spots based on isoelectric point and molecular weight. The 2D gel map helps to compare and match different sets of samples in order to identify isoforms, mutants, and post-translationally modified proteins for statistical analysis. PDQuest software matches and analyzes visualized protein spots among different gels and compares protein intensity between control and experimental gel images. It has powerful, automatching algorithms that identify and accurately match gel protein spots in terms of their intensity and determines if the spots are significantly different.

Several proteins were differentially expressed in our DS model and identified by mass spectrometry. These proteins include aldehyde dehydrogenase, aldolase, alpha enolase, ATP synthase, heat shock cognate 71, and peptidyl-prolyl cis-trans isomerase. The
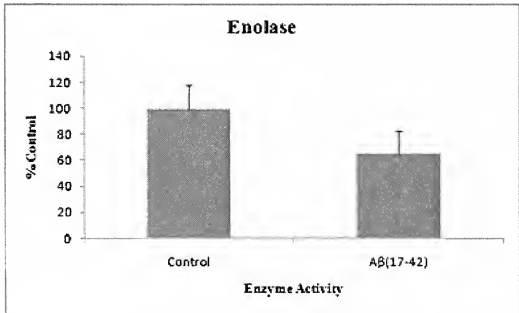

Figure 6.   Enzyme activity of enolase in control synaptosomes and Aβ(17–42) samples. Error bars indicate the SEM for each group measured (n = 6).
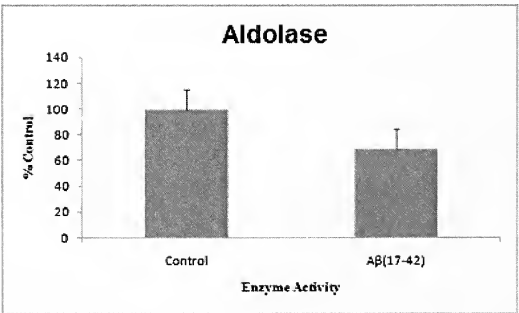

Figure 7.   Enzyme activity of aldolase in control synaptosomes and Aβ(17–42) samples. Error bars indicate the SEM for each group measured (n = 6).

majority of these proteins are directly or indirectly involved in processes related to energy metabolism. We found a reduction in enzyme activity in several enzymes suggesting Aβ(17-42) induced toxic effect contributes to their decreased enzyme activity.

Glucose is the main source of energy in the normal brain (31), and a decrease in ATP production could cause disturbances in the ion and cholesterol homeostasis, cholinergic defects, altered protein synthesis, protein degradation, and synaptic transmission. All of which could be detrimental to the viability of the cell (6). These changes may expose phosphatidylserine to the outer leaflet of the plasma membrane, triggering a loss of phospholipid asymmetry, an early signal of synaptosomal apoptosis (18).

Enolase, a key glycolytic enzyme, is one of the most differentially expressed brain proteins found in human and animal tissues (20). α-enolase catalyzes the dehydration of 2-phosphoglycerate to phosphoenolpyruvate in the glycolytic pathway. This enzyme belongs to a new class of surface proteins that do not possess classical machinery for surface transport, but are transported on the cell surface by an unknown mechanism (19). Enolase has been recently reported to be a moonlighting protein having important roles as a neurotrophic factor (15), a hypoxic stress protein, and a strong plasminogen binding protein (5). α-enolase is one of the most consistently upregulated and oxidatively modified proteins in brain of early-onset AD, amnestic mild cognitive impairment, and late stage AD (6). Taken together, all of these findings suggest that enolase may possess one or more additional functions critical to brain cell survival along with its role in glucose metabolism. Furthermore, enolase could be integral to normal and pathological brain function, and it may possess other functions that are normally necessary to preserve brain function. Loss of enolase's enzymatic activity is a significant factor for AD progression and is consistent with research showing that glycolytic enzymes are functionally altered in neurodegenerative disorders (5,6).

Fructose 1,6-bisphosphate is cleaved to glyceraldehyde-3-phosphate and dihydroxyacetone phosphate via the enzyme aldolase thereby generating ATP (16). Aldolase exists in three isoforms: A, B, and C. Aldolases A and C are preferentially involved in the glycolytic pathway and are mostly expressed in muscle and brain, respectively. Aldolase B plays a role in gluconeogenesis and is typically expressed in the liver (25). Upregulation of this enzyme suggests impaired energy metabolism that could lead to a decrease in overall ATP production. As a consequence, this may lead to a decrease in synapse number and synaptic function thereby promoting memory loss because ATP is very crucial at nerve terminals for normal connections between neurons. Additionally, reduced ATP production may alter glucose and glutamate transporters, cholinergic defects, improper pump maintenance, protein synthesis, disturbances in cholesterol homeostasis, and signal transduction, ultimately leading to cell death and consequently cognitive decline (29).

Aldehyde dehydrogenases (ALDH) are a class of detoxification enzymes that remove excess aldehydes present in the body (Chen et al., 2010). Aldehyde dehydrogenase catalyzes the oxidation of various aldehydes (i.e., carbonyls) to carboxylic acids and is known to play an important role in xenobiotic and endobiotic metabolism (Saini and Shoemaker, 2010). Three classes of ALDH (ALDH1, ALDH2, and ALDH3) have been studied with respect to cytotoxic aldehyde metabolism. Acetaldehyde is considered a neurotoxic product produced during the metabolic pathway of valine and threonine. It is also believed that accumulation of toxic acetaldehyde or aldehyde derivatives could affect the development of the pathogenesis of AD (Kamino et al., 2000). All three classes of ALDH enzymes metabolize HNE and utilize NAD^+ as a cofactor (Picklo et al., 2002). Upregulation of this protein would result in reduced detoxification capacity of the cell and an increase in protein carbonyls demonstrated in Figure 2.

The brain is highly susceptible to oxidative stress and to overcome this vulnerability, the brain has developed networks to combat oxidative stress. One such cellular stress response is heat shock proteins, which protect cells from various forms of stress. Several of these proteins are thermotolerant and resistant to other environmental stresses. Heat shock response has a cytoprotective role in a variety of metabolic disturbances and injuries,

such as hypoxia, epilepsy, stroke, cell and tissue trauma, aging and neurodegenerative diseases (Calabrese et al., 2006). Heat shock proteins serve as molecular chaperones, which exist in various types, among these Hsp32 (also known as heme oxygenase-1), Hsp60, and Hsp72 have been shown to play a protective role in the brain in regard to oxidative stress (Abdul et al., 2006). Heat shock cognate (Hsc71), is an isoform Hsp73, is employed by the cell as a primary defense under unfavorable conditions. Hsc71 is specifically involved in the degradation of proteins with abnormal conformation; it can bind to a specific peptide region and label it for proteolysis and prevent protein aggregation (Castegna et al., 2002). Upregulation of oxidized Hsc71 can result in lowered cytoprotection and an increase in protein aggregation. This can also cause protesomal overload which has been observed in Down syndrome individuals (Yoo et al., 1999).

The α and β subunits of the membrane bound ATP synthase complex bind ATP, ADP, and P_i. The α subunit is involved in the regulation of ATP synthase activity, while the β subunit contributes to catalysis. The ATP synthase complex plays an important role in energy transduction in living cells (Walker et al., 1982). ATP synthase produces ATP by complex rotational movements of its subunits, and coupling the proton gradient generated by the respiratory chain (Sultana, Boyd-Kimball, et al., 2006). ATP synthase dysfunction may decrease the activity of the entire electron transport chain and could contribute to impaired ATP production (Boyd-Kimball, Castegna, et al., 2005). Notably, this is the only enzyme to be down regulated in this DS model. Therefore, ATP will not be generated at normal levels that can alter overall energy metabolism, a highly regarded theory of Alzheimer Disease.

Peptidyl-prolyl cis-trans isomerase (Pin-1) supports the formation of correct disulfide bonds during protein folding. Pin-1 is a chaperone enzyme that catalyzes the isomerization of the peptide bond between pSer/Thr-Pro in proteins, thereby controlling their biological functions including protein assembly, folding, intracellular signaling, intracellular transport, transcription, cell cycle progression and apoptosis (Butterfield et al., 2006).

Pin-1alteration can cause remarkable structural modification, which can affect the properties of targeted proteins. In most of the cells, including neurons, Pin-1 is mostly nuclear and its activity is required for the cell cycle checkpoint of DNA replication. The normal function of Pin-1 includes phosphorylation of cytoskeletal proteins, such as tau. The targeting of the tau protein by an unregulated Pin-1 protein can result in hyperphosphorylation of amino acid residues. Hyperphosphorylation is a component of neurofibrillary tangles, which is observable in DS brain (Schochet et al., 1973) and a hallmark of AD. Several studies have shown that hyperphosphorylation of tau protein may occur due to improper activation of mitotic events in the cell cycle, thereby playing an important role in the progression of Alzheimer's disease (Butterfield et al., 2006).

SUMMARY

The p3 fragment is extensively deposited in DS and observed in AD. Individuals with DS have a trisomy at chromosome 21, the location of amyloid precursor protein. Overexpression of the amyloid precursor protein (APP) is related to the deposition of amyloid in the brain of DS individuals and it appears to be critical to the development of AD in DS individuals. Because of this dementia, the brain regions responsible for thought, memory, and language are affected which leads to further serious cognitive decline and the inability to carry out normal daily activities.

In this study, we identified six biomarkers that were differentially expressed in $A\beta(17-42)$ treated synaptosomes compared to control synaptosomes. The proteins that were found to be upregulated include aldehyde dehydrogenase, aldolase, alpha enolase, heat shock cognate 71, and peptidyl-prolyl cis-trans isomerase. The only protein found to be downregulated was ATP synthase. It has been shown that protein oxidation may lead to protein conformational changes (Subramaniam et al., 1997) and loss of function (Hensley et al., 1995; Kim et al., 2000). Based on this concept, oxidation and successive loss of function of the proteins identified in our study would lead to the disruption of the synapse, neuronal communications, and impairment of energy metabolism. Taken

together, the oxidative stress induced by Aβ(17-42) in synaptosomes in this current study is similar to the oxidative stress induced by Aβ(1-42) found in AD brain (Boyd-Kimball, Castegna, et al., 2005).

Our present findings, suggest the role of Aβ(17-42) as one of the contributing factors in mediating oxidative stress in DS and AD brain leading to neurodegeneration. Protein oxidation observed in our study suggests that oxidative stress may be an early event in the progression of neurodegenerative diseases. The loss of enzyme activity by oxidative modification may contribute to abnormal energy production found in many neurodegenerative disorders. Furthermore, these findings support the role of Aβ(17-42) as a mediator of oxidative stress and a causative agent in the pathogenesis of Down syndrome and Alzheimer's disease.

ACKNOWLEDGEMENTS

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Abstracts of Some Papers Presented at the 2010 Annual Meeting of the Kentucky Academy of Science

Edited by Robert J. Barney

AGRICULTURE

Evaluation of Sunflower (Helianthus annuus L.) Cultivars for Cut Flowers. CHRISTOPHER G. FERGUSON, MARTIN J. STONE, and ELMER GRAY*, Department of Agriculture, Western Kentucky University, Bowling Green, KY 42101.

The sunflower (*Helianthus annuus* L.), an ancient cultivated crop for oilseed production, more recently has gained acceptance as a cut flower in the florist industry. Objectives of the present study were to survey available sunflower cultivars for adaptability to Kentucky growing conditions and for suitability to local florist markets. In 2011, sunflower cultivar trials were conducted at Bowling Green (36.93 N, 86.47 W) and Owensboro (37.78 N, 87.14 W) Kentucky. The randomized complete block design included 18 diverse cultivars and three replications. An experimental unit consisted of 10 plants of each cultivar spaced 30 × 60 cm. apart. Data were collected on seedling emergence, days to flowering, plant height, head diameter, stem diameter, branching, petal color, pollen production, and vase life. Twice weekly harvests of flowering heads began 29 July and continued through mid-September resulting in 9 and 10 harvests in Bowling Green and Owensboro; respectively. Flowering heads were evaluated by both lay and professional groups. The data on plant and head characteristics are being stratified to determine the most adapted cultivars for growing in Kentucky and the ones most acceptable by the florist industry.

Effect of Tassel Removal for Baby Corn Production in Kentucky. CHRISTOPHER G. FERGUSON*, ZHENG WANG, MARTIN STONE, and ELMER GRAY, Department of Agriculture, Western Kentucky University, Bowling Green, KY 42101-3576.

Baby corn (*Zea mays* L.) consists of unfertilized young ears harvested at silk emergence. The 2011 study was a culmination of four successive years of production and evaluation of baby corn at Western Kentucky University (36.93 N, 86.47 W). The objective was to compare the effect of tassel removal on baby corn (BC) production on four cultivars, two field ('N77P-3000GT', 'N68B-3000GT') and two sweet ('Silver Queen', 'Peaches N Cream') corns. Results indicated that tassel removal gave significant increases ($P < 0.01$) of BC ears across harvests (H) and cultivars; however, the effect was not consistent over treatments. For harvests, the difference due to detasseling was highly significant ($P < 0.01$) for H1, significant ($P < 0.05$) for H2 and H3, but not significant ($P > 0.05$) for H4. For cultivars, numerical values were higher for detasseled than non-detasseled treatments in the first three harvests for each cultivar, but significant ($P < 0.05$) only for Peaches N Cream. Quality of BC from both tassel treatments decreased in H3 and H4. Further study is needed to determine economic returns of detasseling BC.

Developing a Biofilter for Remediation of Pesticide Residues in Runoff Water. GEORGE ANTONIOUS, College of Agriculture, Food Science, and Sustainable Systems, Department of Plant and Soil Sciences, Kentucky State University, Frankfort, KY 40601.

Bioremediation is the use of living organisms, primarily microorganisms, to degrade environmental contaminants into less toxic forms. Nine biobeds (ground cavity filled with a mixture of composted organic matter, topsoil, and a surface grass) were established at Kentucky State University research farm (Franklin County, KY) to study the impact of this practice on reducing surface runoff water contamination by residues of dimethazone and trifluralin herbicides arising from an agricultural field. Biofilter systems were installed at the bottom of the slope of specially designed runoff plots to examine herbicides retention and degradation before entering streams and rivers. In addition to biofilter systems, three soil management practices: i) municipal sewage sludge (SS), ii) SS mixed with yard waste compost (SS + YW), and iii) no-mulch rototilled bare soil (NM used for comparison purposes) were used to monitor the impact of soil amendments on herbicide residues in soil following natural rainfall events. Organic amendments increased soil organic matter content and herbicide residues retained in soil following rainfall events. Biofilters installed in NM soil reduced dimethazone and trifluralin by 84 and 82%, respectively, in runoff water that would have been transported down the land slope of agricultural fields and contaminate natural water resources. Biobeds installed in SS and SS + YW treatments reduced dimethazone by 65 and 46% and trifluralin by 52 and 79%, respectively. The use of biofilters in on-farm bioremediation of pesticide residues in surface runoff water might provide a potential solution to contaminated runoff and seepage water arising from agricultural production operations.

Evaluation of Constructed Wetlands for Nitrate and Phosphorus Removal. ERIC T. TURLEY*, and GEORGE F. ANTONIOUS, College of Agriculture, Food Science, and Sustainable Systems, Department of Plant and Soil Sciences, Kentucky State University, Frankfort, KY 40601.

Constructed wetlands are designed and utilized to reduce or eliminate the effect of agrochemicals on water

quality. The use of agrochemicals, such as fertilizers, requires practices for remediation of these environmental contaminants. At Kentucky State University Research Farm, twelve constructed wetland microcosms were established. Six microcosms were filled with river gravel and six with grade #2 limestone. Sweet flag plants, *Acorus calamus*, were planted in six microcosms (nine plants in three river gravel microcosms and nine plants in three limestone microcosms). The plants were observed for growth and performance in the two types of rocks. Water samples were collected at regular time intervals to monitor the performance of the microcosms. Improvement was noted in orthophosphate, nitrate, ammonia, and dissolved oxygen concentrations. The results revealed that microcosms containing limestone, with and without sweet flag, had a 23% reduction of orthophosphate ions compared to microcosms with river gravel with and without sweet flag. The NO_3-N content in microcosms containing limestone and planted with sweet flag was reduced by 42% compared to microcosms containing river gravel and planted with sweet flag. Microcosms containing either limestone or river gravel and sweet flag had increased dissolved oxygen content over microcosms containing either limestone or river gravel and no plants. The NH_3-N content in all microcosms was reduced to near immeasurable amounts.

Half-lives of Endosulfan Isomers on Field Treated Vegetables. KYLA ROSS*[1], GEORGE ANTONIOUS[1], and TEJINDER KOCHHAR[2], [1]College of Agriculture, Food Science, and Sustainable Systems, [2]Department of Biology, Carver Hall, Kentucky State University, Frankfort, KY 40601.

Endosulfan 3 EC, a mixture of α- and β-stereo isomers, was sprayed on field-grown pepper and melon plants at the recommended rate of 0.44 kg A.I. acre^{-1}. Plant tissue samples (leaves and fruits) were collected 1 h to 30 days following spraying and analyzed for endosulfan isomers. Analysis of samples was accomplished using a gas chromatograph (GC) equipped with a mass detector in total ion mode. The results indicated the formation of endosulfan sulfate as the major metabolite of endosulfan sulfite and the relatively higher persistence of the β-isomer as compared to the α-isomer. The initial total residues (α- and β-isomers plus endosulfan sulfate) were higher on leaves than on fruits. On pepper and melon fruits, the α-isomer, which is the more toxic to mammals, dissipated faster ($T_{1/2}$ = 1.22 and 0.95 d, respectively) than the less toxic β-isomer ($T_{1/2}$ = 3.0 and 2.5 d, respectively). These results confirm the greater loss of the α-isomer compared to the β-isomer, which can ultimately impact endosulfan dissipation in the environment. The higher initial residues of endosulfan on pepper leaves should be considered of great importance for timing field operations and the safe entry of harvesters due to the high mammalian toxicity of endosulfan.

Antioxidants and Heavy Metals Content of Hot Pepper. McKENZIE JOHNSON*[1], GEORGE ANTONIOUS[1], and TEJINDER KOCHHAR[2], [1]College of Agriculture, Food Science, and Sustainable Systems, [2]Department of Biology, Carver Hall, Kentucky State University, Frankfort, KY 40601.

Hot pepper accessions that strongly accumulate heavy metals in their edible portions should be regarded as potential source of heavy metal contamination in the food supply. Phenols, ascorbic acid, capsaicin, and β-carotene are some of the classes of naturally occurring compounds having antioxidants activity in hot pepper. However, elevated concentration of heavy metals in hot pepper fruits could expose consumers to potentially hazardous chemicals. The main objectives of this investigation were to: i) to select candidate accessions of hot pepper having high concentrations of phytochemicals for use as parents in breeding for these antioxidant compounds, and ii) assess if hot pepper genotypes that contain great concentrations of capsaicin are also heavy metals (Cd, Cr, Ni, Pb, Zn, Cu, Mo) accumulators. Seeds of hot pepper (*Capsicum chinense*) were collected from Belize, Brazil, Colombia, Ecuador, Mexico, Peru, Puerto Rico, and U.S. and planted in a silty-loam soil. Fruits of PI-640900 (U.S.A.) contained the greatest concentration of capsaicin (1.52 mg g^{-1} fruit) and dihydrocapsaicin (1.16 mg g^{-1} fresh fruit), while total major capsaicinoids (capsaicin and dihydrocapsaicin) in the fruits of PI-438648 (Mexico) averaged 2 mg g^{-1} fruit. PI-152452 (Brazil) and PI-360726 (Ecuador) contained the greatest concentrations of ascorbic acid (1.2 and 1.1 mg g^{-1} fruit, respectively). While PI-438648 (Mexico) contained the greatest concentration of total phenols contents (349 µg g^{-1} fruit), PI-355817 (Ecuador) contained the greatest concentration of β-carotene among the other 63 accessions tested. Variability of these traits might be utilized via plant breeding approaches for their value-added health-promoting characteristics.

An Update on the KSU Pawpaw Breeding Program. KIRK W. POMPER*, SHERI B. CRABTREE, and JEREMIAH D. LOWE, College of Agriculture, Food Science, and Sustainable Systems, Kentucky State University, Frankfort, KY 40601-2355.

The North American pawpaw [*Asimina triloba* (L.) Dunal] is a tree fruit native to the eastern United States, which is in the early stages of domestication. Pawpaw fruit have fresh market appeal for farmers' markets, community supported agriculture, and organic markets, as well as processing potential for frozen pulp production. New high yielding cultivars with excellent fruit quality would assist in the development of a pawpaw industry. Kentucky State University (KSU) serves as the National Clonal Germplasm Repository for pawpaw, and germplasm evaluation is an important research priority. Pawpaw germplasm has been screened for superior fruiting characteristics and trial as new pawpaw varieties.

Pawpaw genotypes in the KSU repository orchards under evaluation are from crosses of current pawpaw varieties or are open pollinated seedlings from a range of genetic sources. Some selections that produced high yields and excellent fruit quality have been selected for clonal propagation (budding onto rootstock) and field trials. Selections that have shown excellent fruit quality and yields include Hi4-1, Hi7-5, H3-120, G4-21, G4-25, G5-23, G6-120, G9-109, and G9-111. Most of the pawpaw advanced selections had similar budding success and vigor to controls and are in field trials. When the genetic diversity of KSU advanced selections were evaluated using Simple Sequence Repeat DNA markers, these selections displayed significant genetic diversity compared to pawpaw cultivars recently released by the PawPaw Foundation breeding effort. KSU advanced selections contain unique pawpaw germplasm that should enhance the genetic base of cultivars if these selections are released to the public.

Ethanol Production Potential from Pawpaw Fruit Agricultural Waste. BRANDON K. MAY*, MICHAEL BOMFORD, KIRK W. POMPER, JON CAMBRON, and TONY SILVERNAIL, College of Agriculture, Food Science, and Sustainable Systems, Kentucky State University, Frankfort, KY 40601-2355.

The finite nature and rate of depletion of fossil fuels has prompted discussion into the production of biofuels to supplement our national energy demands. Government mandates diverting corn or other grain products into cellulosic ethanol production has caused speculation into consequences of a reduced grain supply leading to increased food and livestock costs. Globally, studies are also being conducted to examine the potential useable sugars and starches derived from agricultural waste products ranging from olive mill waste, corn stover, peanut, and fruit waste. The pawpaw [Asimina triloba (L.) Dunal] is a native tree fruit and is a new high-value fruit crop in Kentucky. Pawpaw fruit have fresh market appeal for farmers' markets, community supported agriculture, and organic markets, as well as processing potential for frozen pulp production. After harvesting of the pawpaw fruit many poorer quality fruit remain unused in the orchard. Seed is a byproduct of pulp extraction of the poor quality fruit; the seed is valuable to nurseries, often selling for $10 per pound. Pulp waste from seed extraction has potential for ethanol production. In initial experiments with pawpaw pulp waste from seed extraction, the extracted pulp sugar contents were found to be as high as 19 °Brix or 112.3 g/L and once processed would yield 10.3% alcohol by volume. Using methods devised by UC Davis for sugar to ethanol conversion, biofuel production potential of the pawpaw fruit appears promising from this agricultural waste. The potential for ethanol production and extraction methods for pawpaw pulp will be discussed.

Prime-Ark®45 and Prime-Jan® Primocane Fruiting Blackberry Production Grown Under Organic Culture in Kentucky. JEREMIAH D. LOWE*[1], KIRK W. POMPER[1], SHERI B. CRABTREE[1], JOHN R. CLARK[2], and JOHN G. STRANG[3], [1]College of Agriculture, Food Science, and Sustainable Systems, Kentucky State University, Frankfort, KY 40601-2355, [2]Fruit Culture & Breeding, 316 Plant Science Bldg. University of Arkansas, Fayetteville, AR 72701, [3]Department of Horticulture, N-318 Agricultural Sciences North, University of Kentucky, Lexington, KY 40546.

Primocane-fruiting blackberries are attractive to Kentucky growers because they can be grown organically and have the potential to produce a niche-market crop from late summer until frost. Therefore, locally produced fruit from primocane blackberry selections can be harvested from July until usually October, providing fruit for sale at farmers' markets, community supported agriculture, and organic markets. In June 2010, a blackberry trial was planted at the KSU Research and Demonstration Farm on the certified organic land. The planting contained four replicate blocks each of the selections of Prime-Jan®, a primocane-fruiting selection from the University of Arkansas, and Prime-Ark®45, a newly released primocane-fruiting blackberry. Plants were arranged in a completely randomized design, with four replicate plots each containing five plants of each selection or cultivar (total of 20 plants of each selection or cultivar) in 10-foot plots. This trial was managed with organic practices following the National Organic Program standards. A combination of cultivation, hand weeding, and straw mulch was used for weed control. Drip irrigation was used as needed. Ripe fruit were harvested from the plants twice weekly, Monday and Thursday, from July until October 2011. Harvest period, yield, and berry weight were recorded for all selections. Prime-Ark®45 had higher yield and greater berry size as compared to Prime-Jan®. Warm summer temperatures in 2011 appeared to reduced fruit set on Prime-Jan® as compared to Prime-Ark®45. Prime-Ark®45 appears to be a desirable cultivar well suited to Kentucky growing conditions.

Kentucky State University Pawpaw Processing: An Update. SHERI B. CRABTREE*, KIRK W. POMPER, and JEREMIAH D. LOWE, College of Agriculture, Food Science, and Sustainable Systems, Kentucky State University, Frankfort, KY 40601.

The pawpaw (Asimina triloba) is the largest tree fruit native to the United States and is being grown on a small scale commercially in Kentucky and surrounding states. Pawpaws produce unique fruit with creamy yellow-orange flesh and a flavor resembling a blend of mango, banana, and pineapple. The fruit's short shelf life and difficulty with postharvest handling and storage are impediments to commercial development. Marketing frozen fruit pulp as a value-added product could be one solution to this problem. However, pawpaw fruit pulp extraction is labor-intensive and made more difficult by the row of

large inedible seeds in the fruit, and valuable pulp may be lost through ineffective extraction methods. The objective of this study was to determine if processed pulp recovery rate differs by pawpaw cultivar. Three sets of five ripe fruit each of five pawpaw cultivars (KSU-Atwood, Mitchell, PA-Golden, Sunflower, and Susquehanna) were selected. Fruit were processed using a modified Roma Sauce Maker with a grape spiral and squash/pumpkin screen to separate seeds from pulp and macerate the pulp. Individual fruit and seed weights were measured to determine percent seed. Processed pulp recovery rate varied significantly among cultivars, with Susquehanna, KSU-Atwood, and Sunflower having a greater percentage of pulp recovered from fruit than Mitchell. Susquehanna, KSU-Atwood, and Sunflower had higher fruit weights than PA-Golden or Mitchell. With large fruit weights, a high rate of processed pulp recovery, and high fruit: seed ratio for more efficient processing, the cultivars KSU-Atwood, Susquehanna, and Sunflower are good choices for Kentucky pawpaw growers.

Beneficial Insects Associated with Fall Established Native Perennial Plant Borders. JERMAINE DUNIGAN*, JOHN D. SEDLACEK, and KAREN L. FRILEY, Kentucky State University, Frankfort, KY 40601.

Native perennial plants are ideal for use in sustainable landscapes and are beneficial to the environment because they create habitat for wildlife species including beneficial insects. Attracting beneficial insects using a farmscaping approach can be important in trying to establish sustainable methods of insect pest management in vegetable and fruit crops. Some researchers have suggested that non-crop vegetation such as grasses and floral strips planted in crop field margins can enhance predaceous arthropod and parasitoid populations. Therefore, the objective of this study was to compare several insect groups colonizing newly established perennial borders vs. non-mowed mixed grass/weedy pasture borders. This study was conducted at the Kentucky State University Research and Demonstration Farm in Franklin County, KY. Sixteen plant species, including big bluestem (*Andropogon gerardii*), thimbleweed (*Anemone virginiana*), New England aster (*Aster novae-anglica*), side-oats grama (*Bouteloua curtipendula*), purple coneflower (*Echinacea purpurea*), gray-headed coneflower (*Ratibida pinnata*), rattlesnake master (*Erygium yuccifolium*), common boneset (*Eupatorium perfoliatum*), blue lobelia (*Lobelia siphilitica*), bee balm (*Monarda fistulosa*), switchgrass (*Panicum virgatum*), foxglove beardtongue (*Penstemon digitalis*), hairy beardtongue (*Penstemon hirsutus*), slender mountain mint (*Pycantheum tennuifolium*), little bluestem (*Schizacharium scoparium*), and prairie dropseed (*Sporobolus heterolepis*), were established in 25 m × 2 m border rows replicated three times. Insects were sampled using four 15 cm × 15 cm sticky traps mounted to tobacco sticks in each border row. Lady beetles, solitary bees in the genus *Agapostemon*, soldier beetles (*Chauliognathus pensylvanicus*), and green lacewings (*Chrysops* sp.) were caught in higher numbers in the native perennial border rows than the pasture borders.

Will Mowing of Primocane-fruiting Blackberries Affect Fruit Ripening? KAREN L. FRILEY*, JOHN D. SEDLACEK, KIRK W. POMPER, JEREMIAH D. LOWE, MICHAEL K. BOMFORD, SHERI B. CRABTREE, MARQUITA L. GRAYSON-HOLT, CHRISTOPHER M. WALES, and RACHEL S. HAYDEN, Atwood Research Facility, Kentucky State University, Frankfort, KY 40601.

Blackberry acreage has increased in Kentucky, while demand still often exceeds supply. The need for sustainable production practices is important for small and limited resource farmers as well as organic producers. Primocane-fruiting blackberries will set on both the overwintered canes as well as the current season primocanes. These varieties will produce two crops per pear – the regular summer crop on the floricanes and then a later crop on the primocanes. 'Prime Jim®' and 'Prime Jan®', which are two commercially available primocane-fruiting varieties, were used in this study. Three replicates of each of the two varieties were initially mowed to ground level 6 April, while the second mowing occurred on 24 June. Fruit ripening in Prime Jim plots began to drop off in late August, while fruit ripening remained constant throughout the season in Prime Jan plots.

Stink Bug Species in Organic Blackberries. MARQUITA L. GRAYSON-HOLT*, JOHN D. SEDLACEK, KAREN L. FRILEY, KIRK W. POMPER, JEREMIAH D. LOWE, MICHAEL K. BOMFORD, SHERI B. CRABTREE, CHRISTOPHER M. WALES, and RACHEL S. HAYDEN, Atwood Research Facility, Kentucky State University, Frankfort, KY 40601.

Stink bugs (Hemiptera: Pentatomidae) are pests of blackberries in Kentucky. These insects insert their beak into drupelets to extract the juice and may also leave a foul odor and taste. Consumer demand for damage-free produce means that growers must use safe and effective management tactics for insect pests. Organic blackberry growers require sustainable and environmentally sound production methods to manage these insects. Spring-mowing of primocanes, on primocane fruiting blackberry varieties could avoid stink bug attack and delay fruit set. In 2011, three replicate plots of each of two varieties, 'Prime-Jim®' or 'Prime Jan®' were initially mowed to ground level on 6 April. Three replicate plots of each variety were then mowed a second time on 24 June. Stink bugs were sampled weekly using Florida Stink Bug Traps and hand collection methods. Stink bugs were found across treatments during the 2011 sampling period which extended from 11 July until September 29. Five stink bug species were identified during the period of fruit ripening in the planting. The green stink bug was the most abundant, followed by rice stink bug and then brown, twice stabbed and one-spotted stink bugs at 53%, 16%,

11%, 11% and 11%, respectively. Both hand collection of stink bugs and the use of the Florida Stink Bug Traps resulted in the capture of stink bugs. Although hand collecting required more time, more than twice as many stink bugs were captured compared to the stink bug trap, at 68% and 32%, respectively.

Does a Methyl Salicylate-based Lure Attract Lady Beetles to Blackberries? JUSTINA RIDDICK*, JOHN D. SEDLACEK, KAREN L. FRILEY, and JOY BIRIKE, Atwood Research Facility, Kentucky State University, Frankfort, KY 40601.

Kentucky produces approximately 45 ha of blackberries for a total value of $1,000,000 annually. Demand for locally grown and damage-free blackberries usually exceeds the supply. Developing more sustainable production methods, including the use of beneficial insect attractants, such as a methyl salicylate-based lure, is important for the success of small and limited resource farmers. Eight blackberry plots, including six collaborators, were located in Franklin, Fayette, Scott and Shelby counties in Kentucky. Three plots were certified organic and the other five plots had no pesticides applied. Four sticky traps and posts were placed in all plots and two PredaLure® lures were placed in each of the PredaLure plots. Sticky traps were collected weekly for twelve weeks, placed in labeled ziplock bags and taken to the laboratory where lady beetles were identified using an illuminated magnifier. Total number per species and average number per trap were then calculated. Pink lady beetle, *Coleomegilla maculata*; seven-spotted lady beetle, *Coccinella septempunctata*; Asian lady beetle, *Harmonia axyridis*; parenthesis lady beetle, *Hippodamia parenthesis*; spotless lady beetle, *Cycloneda munda*; orange-spotted lady beetle, *Brachicantha ursina*; twice-stabbed lady beetle, *Chilocoris stigma*; and mildew eating lady beetle, *Psyllobora vigitima-culata* were caught in PredaLure baited sites. Parenthesis and seven-spotted lady beetles were not caught in non baited sites. PredaLure plots had more pink lady beetles, while non PredaLure plots had more Asian, spotless, and mildew-eating lady beetles.

Lady Beetles Associated with Sweet Corn Bordered by Pasture, Buckwheat or Sunflower Borders. JOHN D. SEDLACEK*, KAREN L. FRILEY, MARQUITA L. GRAYSON-HOLT, CHRISTOPHER M. WALES, and RACHEL S. HAYDEN, Atwood Research Facility, Kentucky State University, Frankfort, KY 40601.

Sweet corn, *Zea mays* 'Garrison®', was grown in replicated plots on Kentucky State University's Agricultural Research and Demonstration Farm in Franklin County, KY. Each 25 m × 12 m plot was bordered on each side of its length by a 2 m wide border of unmowed pasture, buckwheat (*Fagopyrum esculentum*), or dwarf sunflower (*Heliathus annuus* var. Big Smile). A randomized complete block design replicating each treatment five times was used and all plots were separated by 25 m. Yellow sticky traps (15 cm × 15 cm) were used to capture lady beetles. Two traps were deployed at canopy height between the edges and equidistant from the ends of each border. Four traps were deployed in each sweet corn plot, one in the center of each plot quadrant. Traps were changed weekly through anthesis. Sticky traps were placed individually in ziplock plastic bags, labeled, and transported to the laboratory for insect identification and enumeration. Pink lady beetle, *Coleomegilla maculata*; Asian lady beetle, *Harmonia axyridis*; spotless lady beetle, *Cycloneda munda*; and seven spotted lady beetle, *Coccinella septempunctata* were caught in this study. The pink lady beetle was the most abundant species in all three border types and the sweet corn plots with 79% and 94% of the lady beetles caught in the borders and sweet corn, respectively. Pink lady beetle numbers decreased in buckwheat from 14 August through 27 August, but increased markedly in sweet corn from 14 August to 20 August, potentially indicating movement into the sweet corn.

Economics Systems, Role of Government, and Agriculture. STEPHEN A. KING, Department of Agriculture, Western Kentucky University, Bowling Green, KY 42101, Stephen.King2@wku.edu.

The role of government in U.S. agriculture is in great part defined by the Farm Bill. The current U.S. Farm Bill is referred to as the "Food, Conservation, and Energy Act of 2008" and its provisions have far reaching impacts over a broad range of constituents. It is set to expire in the year 2012, at which time a new Farm Bill is expected to be enacted. In addition to traditional commodity programs that directly impact the decisions of farmers, the legislation impacts the development of organic agriculture and biofuels markets, conservation of natural resources, nutrition and rural housing programs, agricultural research, among other areas. As a society, how do we decide what should and should not be included in the Farm Bill? Do we have any set of criteria for deciding the role of government in agriculture? How does current and past farm legislation influence the economic system of the U.S.? The work presented addressed these questions. It has been hypothesized that our socio-economic values influence the political process and thus the legislation that is developed and in turn the role of government in agriculture and therefore the economic system. In general the research suggests that current and past agricultural legislation moves our economic system toward the direction of centrally planned capitalism, that is agricultural resources are predominately owned by the private sector but their allocation is strongly influence by governmental policy. A set of criteria were proposed for guiding the role of government in agriculture.

Characteristics of and Reasons Why Farmers Choose Off-farm Work. CAITLIN N. CARTER, Department of Agriculture, Western Kentucky University, Bowling Green, KY 42101. caitlin.carter472@topper.wku.edu.

In recent years, farmers in the United States have been seeking off-farm work to supplement farm operations income. In many cases, income provided by off-farm work is the largest component of the farm household income. Previous research by the United States Department of

Agriculture – Economic Research Service suggests that the extent to which producers rely on off-farm income is dependent upon farm size and the type of enterprise. Results of this study reveal the most prominent characteristics of those farmers who choose off-farm work, the reasons why they choose off-farm work, and the extent of income that off-farm work provides to various categories of farm households.

ANTHROPOLOGY AND SOCIOLOGY

Acculturation and Body Weight Status of Chinese Immigrants in Kentucky. CECIL BUTLER*, LINGYU HUANG, and CHANGZHENG WANG, Human Nutrition Program, Kentucky State University, Frankfort, KY 40601.

Traditional Chinese diets are rich in vegetables and fruits and obesity is less prevalent among Chinese people. Immigrants adapt to American diets and behavior patterns. The objective of this study was to assess the acculturation and body weight status of Chinese immigrants in Kentucky. Thirty Chinese American immigrants were recruited to participate in the study at a large community event. The subjects were asked to fill out a questionnaire before they were given a free analysis of their body composition (body fat %) with a Tanita TBF-521 body composition analyzer. Body mass indexes were calculated from the body weight and height measured on-site. 84% of the participants were within normal body weight range with only 10.5% in the overweight and 5% in the obese category. Close to 50% of them speak Chinese and English about the same, but 28% speak mostly English. 46% of them read better in Chinese and speak mostly Chinese at home, but another 46% read better in English and speak mostly English at home. Only 38% speak Chinese only with friends, 53% speak only English or mostly English to friends. 30% think in mostly Chinese, 23% think in Chinese and English about the same, but 45% think in mostly English. 54% watch TV mostly in English with 30% do so mostly in Chinese. Over 73% listen to radio mostly in English with none listening to radio in Chinese. 54% identify themselves as Chinese American with 23% identifying with Chinese or American. 38% have mostly Chinese friends and 46% have some non-Chinese friends. 85% either agree or strongly agree with the statement that "I think of myself as being U.S. American." 69% are proud or very proud of their Chinese background. 69% eat mostly Chinese foods. 58% celebrate Chinese holidays most of the time. In conclusion, there are different degrees of acculturation among Chinese immigrants but the effect on their body weight status was not clear due to the limited number of subjects in this study.

Body Weight Perception and Willingness to Adopt Healthy Eating and Activity Behaviors among Kentucky Adults. ERICA COLEMAN*, LINGYU HUANG, CECIL BUTLER, and CHANGZHENG WANG, Human Nutrition Program, Kentucky State University, Frankfort, KY 40601.

Visitors to the 2011 Kentucky State Fair were recruited to fill out a questionnaire before they were given a free analysis of their body composition (body fat %) with a Tanita TBF-521 body composition analyzer. 60% of overweight men considered themselves normal and 77% of obese men considered themselves only overweight. 15% of normal weight women considered themselves overweight, but only 21% of overweight women considered themselves normal and 51% of obese women put themselves in the overweight category. 80% of the participants would choose vegetables or fruits and nuts for snacks but 30% of the obese group would choose chips for a snack. 70% of the participants would learn to prepare vegetable dishes on their own but only 14% would do so by attending free workshops. 72% of participants were willing to add physical activities to their daily life such as walking but only 10% were willing to join a free club for exercise and 10% of the obese is willing to pay for an exercise program. 60% of the obese group was willing to cut soft drinks and 50% of the normal weight and overweight individuals were willing to drink water only. In conclusion, self-perception of body weight tended to lower the severity of weight problems in both men and women. Furthermore, self-perception of body weight status could affect the willingness to adopt healthy eating and activity behaviors.

BOTANY

Effect of Natural Plant (*Cocos nucifera*) Derived Oil on Ulcerative Colitis in a Murine Model. PRANAV CHANDRA*, and NILESH SHARMA, Ogden College of Science and Engineering, Department of Biology (TCNW), Western Kentucky University, Bowling Green, KY 42101.

Ulcerative colitis (UC) is a chronic disease of the colon or large intestine that causes inflammation and ulceration (tiny open sores) of the inner lining of the colon and rectum. Ulcerative colitis can occur in all areas of the colon. In patients with ulcerative colitis, the body's immune system over-reacts and body mistakes food, bacteria, or other internal materials in the colon for an invading substance and it signals the immune system to attack the material, thus irritating the colon. This irritation triggers a flare of ulcerative colitis symptoms likewise bloody, pus or mucus filled stools, diarrhea, cramping, abdominal pain and bloating. Highest incidences are seen in the United States, Canada, the United Kingdom and Scandinavia. Since the etiology of UC remains unclear, successful treatment strategies targeting large sections of affected population have not been found. UC is currently treated with medications that include a combination of anti-inflammatory, immunosuppressive and antibiotic drugs with limited remission and significant episodes of side effects; often patients become refractory and seek an alternative therapy. Lack of efficacious drugs to treat patients with different forms of inflammatory bowel disease underscores need for the development of a new and effective alternative therapy. Currently, the role of saturated fatty acids on human health is being revisited, and this issue is drawing significant attention specifically

in inflammatory and metabolic disorders. Effects of medium-chain saturated fatty acids (MCFAs) - like lauric and caprylic acid- have been little studied, and thus drawing much attention. Natural coconut (*Cocos nucifera*) oil is a rich source of MCFA, main constituent being lauric acid: a 12C-chain of fatty acids. Traditionally, coconut oil has been used as cooking oil in several parts of India and other Asian countries. Lauric acid converts to the fatty acid monolaurin in our body and has adverse effects on several microorganisms including bacteria, fungi, yeast and enveloped viruses. Lauric acid is one of the main components of human breast milk, and boosts immune system of children during infancy. Limited knowledge of inflammatory conditions coupled with a narrow range of therapeutic options necessitates investigating the role of natural products. Therefore, the present study focuses on the anti-inflammatory role of natural fatty acids derived from *Cocos nucifera* in the murine model of ulcerative colitis.

Genetic Diversity in Kentucky Spicebush Populations Using Simple Sequence Repeat Markers. RE'GIE SMITH*, KIRK W. POMPER, JEREMIAH D. LOWE, JACOB BOTKINS, and SHERI B. CRABTREE, College of Agriculture, Food Science, and Sustainable Systems, Kentucky State University, Frankfort, KY 40601-2355.

Spicebush (*Lindera benzoin* L.) is an aromatic small native shrub that grows in the moist, understory areas of Appalachia and has potential as a new niche crop for small farmers. Native Americans and early settlers used this plant traditionally as a tea. The berries can be used for jam and spicing of foods, and may have health benefits including antioxidant compounds. Native spicebush patches also can serve an important role in forest ecosystems in terms of fruit production for animals, soil erosion control, and enhancing insect biodiversity. Spicebush may serve to hold ecological niches by outcompeting invasive plants compared to those in unchallenged areas. Genetic diversity of native spicebush populations in Kentucky has not been examined. The objective of this study is to determine the genetic diversity in spicebush populations in Kentucky using simple sequence repeat (SSR) DNA marker systems. Leaf samples were collected from 20 spicebush plants in the forests at the Kentucky State University Environmental Education Center (EEC) and at a location near the Kentucky River. DNA was extracted using the DNAmite Plant Kit. Primers A7, A115, B105, and B122 were used to amplify SSR products that were separated with a 3130 Applied Biosystems capillary electrophoresis system. The software program Power Marker was used to examine genetic relationships among genotypes. The SSR markers generated showed genetic variation among the spicebush genotypes. A number of selections with unique genotypes will be sampled and propagated for study in the KSU germplasm collection for potential cultivar development.

Pawpaw Patch Genetic Diversity and Clonality and its Impact on the Establishment of Invasive Species in the Forest Understory. JACOB BOTKINS*, KIRK W. POMPER, JEREMIAH D. LOWE, and SHERI B. CRABTREE, College of Agriculture, Food Science, and Sustainable Systems, Kentucky State University, Frankfort, KY 40601-2355.

The pawpaw (*Asimina triloba*) is a native understory tree of 25 states of the east and midwest United States. Pawpaw's ability to compete with local invasive species in Kentucky has not been examined. The objectives of this study were two-fold: to determine the genetic diversity and clonality displayed in seven native pawpaw patches located at the Kentucky State University Environmental Education Center (KSU-EEC), the Kentucky River, Cove Spring Park, and the KSU Research and Demonstration Farm in Franklin County, using microsatellite markers; and to determine if patches reduced the incidence of invasive species. Twenty-five trees from seven patches in the four different locations were sampled for genetic analysis. Leaf samples were extracted using the DNAmite Plant Extraction Kit and products from four microsatellite loci were analyzed using a 3130 Applied Biosystems capillary electrophoresis system. String grids were created and invasive plants counted in three 10-meter squares in each of the patches and control plots outside of each patch. The number of plants for each invasive species within pawpaw patches was counted and compared to a control plots. Pawpaw patches displayed high genetic diversity among populations. Japanese honeysuckle (*Lonicera japonica*), garlic mustard (*Alliaria petiolata*), winter creeper (*Euonymus fortunei*), and colts foot (*Tussilago farfara*) were found in most locations; however, there was no significant difference in the incidence of invasive plants between the patches and the control plots. Pawpaw stem density may be important in the incidence of invasive plants within patches.

CHEMISTRY

Synthesis of Homoleptic Nickel (II) Complexes and Examination of their Coordination Dynamics in Solution. LAURA BISHOP*[1], DAVINDER KUMAR[2], CRAIG A. GRAPPERHAUS[2], and CHRISTOPHER S. MULLINS[1], [1]Division of Natural Science, Campbellsville University, Campbellsville, KY 42718, [2]Department of Chemistry, University of Louisville, Louisville, KY 40292.

In this study, we have begun to examine the structural dynamics for a group of homoleptic nickel (II) complexes. All of the tridentate ligands have one fairly acidic proton attached to an oxygen or nitrogen donor atom that ligates the metal in the first coordination sphere. The ligand 2-(salicylideneamino)-1-hydroxyethane (H_2-SALAHE) has been studied extensively for a variety of applications, including the synthesis of single-molecule magnets derived from cluster compounds. A previous publication of the homoleptic Ni(II) complex of this ligand reported the single-crystal X-ray structure, wherein the nickel ion was found to be octahedrally coordinated by two of the ligand molecules. Our recent studies with this complex suggest

that the complex undergoes a coordination number change in solution to give a four-coordinate nickel complex. This complex has been found to give green crystals reminiscent of the octahedral structure upon recrystallization. Future work will utilize several spectroscopic techniques such as UV-Visible absorption, EPR, and NMR, etc. in order to study the fluxional nature of these complexes in solution.

COMPUTER AND INFORMATION SCIENCES

Statistical Analysis of Microarray Gene Expression Data from a Mouse Model of Toxoplasmosis. SHRIKANT PAWAR*, CHERYL D. DAVIS, and CLAIRE A. RINE-HART, Department of Biology, Bioinformatics and Information Science Center, Western Kentucky University, Bowling Green, KY 42101.

Toxoplasmosis, caused by the protozoan parasite, *Toxoplasma gondii*, is a major cause of morbidity and mortality in patients with AIDS and an important cause of miscarriage, stillbirth and congenital disease in newborns. Previous studies have provided evidence that dietary supplementation with vitamin E and selenium is harmful during experimental toxoplasmosis in mice, whereas a diet deficient in vitamin E and selenium results in decreased numbers of tissue cysts in the brain and dramatically reduced brain pathology. The overall goal of the present study was to determine the impact of dietary supplementation with antioxidants on gene expression in the brains of non-infected mice and in mice infected with *T. gondii* using microarray analysis. RNA was isolated from the brains of C57BL/6 mice, and an Agilent Oligo Whole Mouse Genome Microarray (Agilent Technologies, Inc.) was performed. A total of 48 chips were normalized by Z ratios and the Data Driven Harr Fisch Normalization methods. Differentially expressed genes were identified by applying thresholds to identify significant values and the results were compared between the normalization methods. These differentially expressed genes and their respective fold change ratios were used in Ingenuity Pathway Analysis (IPA) software to analyze the pathways involved with these genes. The identified pathways associated with differentially expressed genes are very important in determining the impact of dietary supplementation with antioxidants on gene expression in the brains of mice infected with *T. gondii* and specific alterations of those pathways can help us in reducing the harmful effects of the same in future. Support from the National Center for Research Resources NIH Grant Number 2 P20 RR-16481 and from the WKU Bioinformatics and Information Science Center is gratefully acknowledged.

ECOLOGY AND ENVIRONMENTAL SCIENCE

Assessing Kentucky State University's Recycling Program. RE'GIE SMITH*, RODNEY RIPBERGER, BRANDAN BURFICT, DUSTIN HODGES, and JOHN D. SEDLACEK, Masters of Environmental Studies Program, Kentucky State University, Frankfort, KY 40601.

The purpose of this study was to conduct a recycling audit of six buildings on Kentucky State University's (KSU) campus and to conduct a survey of the attitudes, perceptions and knowledge of faculty, staff and students about recycling on campus. A six-week audit was conducted of waste from two academic buildings, one administrative building, the student center, and one male and one female dormitory for a total of six campus buildings. Recycling bins were located in the six buildings and their locations were documented on maps. We collected a total of 815 lbs of waste; of the waste that was collected 361 lbs (44%) could have been recycled. Thirty six percent of the recyclables was plastic, 28% was paper and 24% was cardboard. The survey revealed that students recycle less compared to the faculty and the staff. The faculty use the recycling bins an average of 1.32 times per day, staff use the bins an average of 1.47 times per day, and the students use the bins an average of 0.66 times per day. The survey respondents' answers showed that they believe recycling is important and almost 97% are willing to help KSU recycle more. In fact, 81% of the respondents recycled prior to life at KSU, and only 3% consider themselves to not be "green," or to not take actions to promote a healthy environment. The census of students, faculty, and staff provided data that will help make recommendations for the KSU recycling program.

Winter Management of an Invasive Species, Garlic Mustard, *Alliaria petiolata*, in Wooded Habitat. JACOB BOTKINS*, RUSSELL WILLIAMS, ADAM GER-UGHTY, and JOHN D. SEDLACEK, Masters of Environmental Studies Program, Kentucky State University, Frankfort, KY 40601.

Garlic mustard, *Alliaria petiolata*, is a biennial cool-season plant growing 0.5–1 m tall. It is an aggressive competitor for resources excluding native plants from their habitats. This plant is shade tolerant allowing it to invade mature woodlands, where it shades out native understory flora and produces allelopathic compounds inhibiting seed germination of other species. It is threatening the federally endangered Braun's rockcress (*Arabis perstellata*) known only from Franklin, Owen and Henry counties. The objective of this research was to quantify two methods of winter management of garlic mustard at the Julian Savanna State Nature Preserve in Franklin County, Kentucky. Hand removal, a 2% glyphosate solution and untreated control treatments were used. Plots were 1 m² in area. A thatching rake was used to hand weed while a hand held 0.5-liter sprayer was used to apply glyphosate to each plot. A digital camera was used to photograph each plot 1.5 m overhead before plot treatment on 16 February. Each plot was photographed nine weeks after treatment and weed control quantified using the NIH ImageJ program (U.S. National Institutes of Health). There was a 22% and 24% increase in garlic mustard and purple deadnettle foliage in glyphosate treated and hand weeded plots, respectively. There was >722% increase in garlic mustard and purple deadnettle coverage in untreated plots. Thus, a single application of glyphosate in mid-February or hand

weeding/surface tilling reduces, but does not eliminate, garlic mustard and purple deadnettle in wooded areas.

White Tailed Deer in Frankfort, Kentucky: Population Assessment and Implications for the Community. JON CAMBRON*, TERRELL HOLDER, MARK RASCHE, KIAH RODRIGUEZ, MIKE WARD, and JOHN D. SEDLACEK, Masters of Environmental Studies Program, Kentucky State University, Frankfort, KY 40601.

A spotlighting assessment of the population of white-tailed deer was conducted in five Frankfort city parks. Two to four replications were done on each park. Cove Spring Park's population was estimated at 24, Capitol View Park - 77, Fort Hill Park - 76, East Frankfort Park - 6, and Juniper Hills Park - 0. Based on a calculated estimate of deer/mi², Cove Springs and East Frankfort densities fell within the range of expected density based on mean densities of adjacent counties; Capitol View and Fort Hill did not. This could be because surveys of Capitol View and Fort Hill were not accurate or the densities are in fact much higher than expected in this region. To supplement the population survey, we did an informal assessment of the forest understory in three of the surveyed parks and looked at deer-vehicle collisions as a proxy for density. Understory vegetation was limited to a small number of species dominated by bush honeysuckle (*Lonicera* spp.) and very few tree saplings of any species were observed. In Fort Hill Park, the understory was essentially non-existent. There were 851 deer-vehicle collisions between 1 January 2001 and 31 December 2010, mostly occurring in October, November and December. The collision count over ten years, looking at only November, suggests a two or three year deer population cycle. The census combined with the informal vegetation assessment and incidence of deer-vehicle collisions in November suggests that deer in Frankfort may be approaching ecological carrying capacity.

Citizen Awareness of Invasive Plant Species in Kentucky. JOHN D. SEDLACEK*, ADAM GERUGHTY, JACOB BOTKINS, and RUSSELL WILLIAMS, Masters of Environmental Studies Program, and MARA MERLINO, and TIERRA FREEMAN, Psychology Department, Kentucky State University, Frankfort, KY 40601.

Non-native invasive species are one of the primary threats to biodiversity. Public support for invasive species management is critical to the success of such projects. Additionally, understanding the public's knowledge, attitudes and perceptions can assist with the development of outreach and educational activities. In order to assess the level of understanding of the invasive plant species threat, attitudes towards invasive species management and demographic factors influencing such attitudes, a questionnaire survey of 400 randomly selected members of the public in the greater Louisville, Lexington, Frankfort and Bowling Green metropolitan service areas was conducted. We developed a survey that determined an elementary level of awareness of invasive plants, people's understanding of

what is native vs. non-native, why these concepts matter, and if respondents are motivated to assist in invasive species removal. The survey was administered via Survey Monkey. Surprisingly, only 4.5% of the population invited to participate in the survey actually responded. Thus, drawing major conclusions from the data would not be advisable. However, the low response indicates that the vast majority of citizens are unaware of, or don't care about, the potential economic or ecological consequences of invasive species establishment. We provided all survey information to the Kentucky State Nature Preserves Commission who hopefully will be able to further address educational issues concerning invasive species and how to better market those messages in Kentucky.

GEOLOGY

Nutrient and Fecal Microbe Assessment of the Water Quality of Tates Creek, Madison County, Kentucky. KRISTOPHER H. CARROLL*, and WALTER S. BOROWSKI, Department of Geography and Geology, Eastern Kentucky University, Richmond, KY 40475.

Tates Creek is a significant tributary to the Kentucky River that has shown high levels of microbial and nutrient pollution. We sampled the waters of Tates Creek comprehensively by occupying 25 stations along its 13-mile length, collecting stream water at the confluence of major tributaries from its headwaters to the Kentucky River. Samples were collected four times between May and August 2011 during dry periods as well as immediately after rainfall events. We measured ammonium (NH_4^+), nitrate (NO_3^-) and phosphate (PO_4^-) concentrations using colorimetry. Microbial samples were measured for total coliform and *Escherichia coli* using IDEXX Colilert-18 media. Background levels of NH_4^+, NO_3^- and PO_4^- are typically ~0.2 ppm, 13 ppm, and 1.0 ppm, respectively. Nutrient concentrations generally increase during rainfall events, presumably because nutrients are flushed into the stream. Background counts of *E. coli* are typically ~100 cfu/mL but microbe counts reached 1,000–2,419 cfu/mL immediately following rain events. A sewage treatment plant exists approximately two miles from the headwaters and noticeably affects water quality. Nutrient concentration, especially NH_4^+ and PO_4^-, are markedly increased at the plant's outflow. These nutrients then decrease steadily in concentration downstream to background levels. In contrast, fecal microbe counts are high upstream from the plant, but fall to near-zero levels at its outflow, and then increase anew downstream. The treatment plant went offline on 19 July 2011, so we will be able to assess any changes in water quality and stream health in the future.

Suspended Sediment Concentration in the Brushy Creek Watershed, Kentucky. TYLER A. WADE*, and WALTER S. BOROWSKI, Department of Geography and Geology, Eastern Kentucky University, Richmond, KY 40475.

Suspended sediment concentration (SSC) can be used as a proxy for environmental health of stream water. For example, large sediment loads can cause harm to aquatic

life and are a mechanism for introducing and transporting fecal microbes. We measure SSC of the Brushy Creek watershed, located in Rockcastle, Pulaski, and Lincoln Counties, where the Eastern Kentucky Environmental Research Institute (EK-ERI) has been conducting an assessment of the watershed. Two auto-sampling units were placed in Brushy Creek to collect water samples for determination of SSC. The units collect samples every 14 hours for a two-week period, then samples are retrieved for analysis, and new sample bottles are loaded into the auto samplers. Sediment sampling has been in progress since January 2011 and will continue until November 2011. We measure sediment transport during dry, wet, and storm periods. Retrieved samples are brought to the laboratory where sediments are filtered and weighed to determine SSC. The SSC data have been evaluated along with records of rainfall events, as recorded by the UK Agriculture weather station located in Somerset, KY. Due to operational difficulties with our water and sediment samplers, we have only collected intermittent data, however, rainfall events seem to be correlated with increased SSC.

The Micro- and Macro- Faunal Diversity of a Devonian Dysaerobic Environment. LARRY TACKETT*, KARA WELLS, and CHARLES E. MASON, Department of Earth and Space Sciences, Morehead State University, Morehead, KY 40351.

This study examined the fauna contained in the type section of the Three Lick Bed of the Ohio Shale (Upper Devonian), which is located in Rowan County, Kentucky. The Three Lick Bed separates the underlying Huron Member from the overlying Cleveland Member of the Ohio Shale. The unit is 3.42 meters thick and is composed of three greenish gray shale beds separated by two intervening black shale beds. The three greenish-gray shale units were hypothesized to be deposited under dysaerobic conditions and thus the focus of this study. To date slightly over 200 kilograms of samples have been processed for macrofossils and 90 kilograms for microfossils. The samples were broken down using the kerosene technique and washed through a nested set of U. S. standard sieves, a #20 for macrofossils and a #100 for microfossils. The residue caught on the #100 sieve underwent heavy liquid separation and both the heavy and the light fractions were examined for microfossils. All picking, sorting, and identification of fossils were conducted under a binocular microscope. The results of this study support our hypothesis that the greenish gray shale units of the Three Lick Bed were deposited in a dysaerobic environment. Evidence supporting this conclusion includes the following: 1) a low diversity macro invertebrate fauna of 15 species, 2) of the 532 specimens identified nearly all were juveniles, 3) the fauna was dominated by mollusks, 12 out of 15 species, and 4) all macro invertebrates except *Lingula* were preserved as pyretic internal molds. Overall, benthic foraminifera dominate the microfossil fauna in both diversity and abundance, followed by ostracodes in terms of abundance. The macrofossil fauna is dominated by ammonoids being the most diverse (with four species) and a low-spired gastropod being the most abundant (198/532).

HEALTH SCIENCE

Procedure for Preparing Purple Sweet Potato Powder. LINGYU HUANG*, CECIL BUTLER, and CHANGZHENG WANG, Human Nutrition Program, Kentucky State University, Frankfort, KY 40601.

Purple sweet potato has health promoting properties. Purple sweet potato powders currently on the market are made by grinding the raw material and sun-drying the precipitates, or grinding of sun-dried slices of the sweet potato. Recent research indicates that such processes lead to significant loss of antioxidants. Our objective was to develop a process that better preserves the antioxidants when the powders are produced. Purple sweet potatoes were obtained from a North Carolina farm. The whole sweet potatoes were steam-cooked at 200°F for 45 min before they were skinned and mashed. The mashed material was dried in a forced air-drying oven at 60°C or 80°C. After drying the materials were crushed and ground into powder in a Hobart grinder. Cooking of the whole sweet potato avoided the activation of enzymes so the damage of the antioxidants would be reduced. The drying process resulted in 71% loss of weight. The skin accounted for 4.5% of the total weight. The drying temperature was critical. At low temperature, the materials would spoil and mold would grow, rendering the materials useless. At high temperature (100°C), browning of the materials occurred potentially damaging the nutrients. Drying process did not significantly reduce the total phenolic content of the powder.

PHYSIOLOGY AND BIOCHEMISTRY

Regulation of EMT Proteins in Breast Cancer Cell Lines. MARY WIECHART*, JACKIE JANSEN, ARIELLE MARASLIGILLER, HILLARY RESTLE, SHANE MULVIHILL, STEFAN SIWKO, and JULIA CARTER, Wood Hudson Cancer Research Laboratory, Newport, KY 41071.

During 2011, 232,620 new breast cancer cases are predicted in the US and 39,970 breast cancer deaths. This high mortality rate is due to tumor metastasis. To metastasize, breast cancer cells must undergo epithelial-mesenchymal transition (EMT), a process that disaggregates the epithelium, reshapes it for movement, and requires biochemical re-programming. Slug and p21 activated kinase (Pak 1) are two proteins that are increased during EMT. Another protein, eukaryotic initiation factor 4E (eIF4E), is elevated in breast cancers. Elevated eIF4E function selectively enhances the translation of mRNAs with long, highly structured untranslated regions (UTRs) such as Slug and Pak 1. We hypothesized that elevated eIF4E function in breast cancer cells may enhance translation of Slug and Pak 1, thereby promoting EMT. To test this hypothesis we examined protein expression in western blots of lysates from 6 breast cancer cell lines with different estrogen, progesterone, and Her 2 receptor status. Since MDA 231 breast cancer cells

expressed all three proteins, are negative for all three receptors and are reported to be the most invasive breast cancer cell line, we used this cell line to determine if knock down of eIF4E by siRNA transfection would alter expression of these EMT associated proteins. We found a slight reduction in Slug expression in MDA 231 cells with reduced eIF4e but a slight increase in Pak 1 expression. Although these data are preliminary, they do not support our hypothesis that eIF4E regulates Slug and Pak 1 expression and possibly EMT in breast cancer.

Two Germline Variants of the *TGFβR1* Gene are Associated with Initiation, Progression and Clinical Outcome of Colorectal Cancer. HILLARY RESTLE*[1], SHANE MULVIHILL*[1], JONATHAN BENDER[1], KEVIN MURRAY[1], JESSICA SHAW[1], BRIANA VOGT[1], ROBERT SHIELDS[1], BRUCE COLLIGAN[1], JAMES DEDDENS[2], LARRY DOUGLASS[1], JAMES SCHAEPER[1], and JULIA CARTER[1], [1]Wood Hudson Cancer Research Laboratory, Newport, KY 41071, [2]University of Cincinnati, Cincinnati, OH 45221.

Germline variations of transforming growth factor beta (TGFβ) are associated with tumor initiation and progression, especially in bladder, breast, ovarian, kidney, and lung cancer. Colorectal cancer (CRC) is the second leading cause of cancer-related deaths in the United States. There will be over 140,000 people diagnosed with CRC this year and nearly 50,000 CRC related deaths. CRC is frequently diagnosed in the later stages due to the non-specific symptoms in its early stages, further emphasizing the need for genetic biomarkers. Two germline variants in the *TGFβR1* pathway were analyzed via capillary electrophoresis in 233 cases and 219 controls to determine if their incidence affected the prevalence and stage of cancer. We hypothesized that these variants could be significant factors in predicting initiation, progression, and growth in CRC. We found that patients with *Int7G24A*, a single nucleotide polymorphism in the intron 7/exon 7 boundary of the *TGFβR1* gene, had a significantly higher incidence of CRC as compared to non-cancer controls. *TGFβR1*6A*, a nine base pair deletion in exon 1 of the *TGFβR1* gene, was not associated with increased CRC incidence but was associated with adenoma patients that did not progress to advanced CRC. This suggests that *TGFβR1*6A* may have a protective effect. Patients with carcinoma-in-situ (CIS) or CRC stages 1-4 showed a significantly increased incidence of the *Int7G24A* variant as compared to non-cancer controls and patients who never progressed beyond adenoma. This discovery indicates that *Int7G24A* could be a biomarker for identifying patients at a higher risk for developing CRC.

SCIENCE EDUCATION

Sharing Ideas About Assessing Student Learning. JOHN G. SHIBER, Division of Nursing, Biology & Allied Health, Big Sandy Community & Technical College, Prestonsburg, KY 41653.

An emerging national policy of holding post-secondary educators more strictly accountable for student learning has many scrambling to re-evaluate their teaching strategies and the assessment parameters they employ. It is an overwhelming challenge because, as colleges increasingly become like businesses and treat their students like clients, student attitudes toward learning are undermined by an equally strong if not stronger one of entitlement, irrespective of how much or little they apply themselves in their studies. This paper discusses the consequent need for an increased number of parameters, besides testing, to help assess student learning in the sciences such as those shown in studies by this investigator to be beneficial: pre-/post-testing, class attendance, in-class writing assignments immediately after reading articles or watching videos on scientific topics, active individual involvement and course-appropriate extra credit opportunities in and beyond the classroom, end-of-semester student opinion questionnaire on course, etc. An argument for establishing continuity among teachers within each science discipline to follow the same assessment guidelines will also be presented.

ZOOLOGY

Measurement of Differential Acid Concentration Along the Developing Gastrointstinal Tract of Tadpoles with an Improved pH Microprobe. SARAH E. CROSS, and RICHARD D. DURTSCHE, Department of Biological Sciences, Northern Kentucky University, Highland Heights, KY 41099.

Digestion and assimiliation of foodstuffs in vertebrates is often dependent upon changes in the chemical environment along the gut depending on the level of food decomposition and the optimality of conditions for enzyme activity. Low pH in the stomach can chemically breakdown food while activating pepsin protein enzymes. Other areas of the gut (e.g., colon) could show lower pH levels that would indicate plant fermentation and the release of volatile, short-chain, fatty acids. Measurements across developmental stages can document ontogenetic shifts in acid concentrations in the gut suggesting upregulation of digestive activites within the gastrointestinal (GI) tract. As free living vertebrates, tadpoles (anuran larvae) undergo developmental changes in the formation of the GI tract, but they are also consuming foods as might an adult vertebrate. Functional changes in digestive processes in tadpoles can therefore be responses to either maturation of the system or a response to the varied foods consumed. Previous research in our lab has shown changes in pH across the GI tract, suggesting digestive processes similar to adult vertebrates. Our continued investigations of differential acid concentrations along the gut has resulted in the design and fabrication of an improved pH microelectrode with a built-in micro reference electrode. We have been testing this new microprobe on locally collected Green frog (*Lithobates clamitans*) tadpoles, where after dissection, various regions of the GI tract were measured for changes in pH. In testing our solid-state microelectrodes with tip diameters of $=10$ μm against standard pH solutions, we maintained precision of $R^2 = 0.95$.

Beyond mtDNA: Morphology and Nuclear Gene Flow Suggest Taxonomic Oversplitting in the Ringneck Snake *Diadphis punctatus*, FRANK M. FONTANELLA, Department of Biology and Chemistry, Morehead State University, Morehead, KY 40351.

Being able to efficiently and accurately delimit species is one of the most basic and important aspects of biology because species are the fundamental unit of analysis in biogeography, ecology, and conservation. This delimitation may be hampered by variation within and between populations making it difficult to determine whether populations have evolved into independent evolutionary units. Recently there has been a resurgence in species delimitation beyond traditional morphological and mitochondrial data that incorporates species distribution modeling and nuclear data to assess ecological divergence and levels of gene flow between populations. Using the Northeastern ringneck snake *Diadophis punctatus edwardsii* as a model, I expanded upon previous work by combining 24 external meristic characters from 300 museum samples, species distribution models generated from 19 climatic variables derived from 415 unique locality data points, and 10 microsatellite loci from 288 individuals, to test whether the mtDNA clades represent distinct evolutionary units. The PCA and CVA analysis of the meristic data failed to recover significant differences between the two mtDNA clades. Demographic analysis of the mtDNA data depicts rapid population expansion of the northern clade that corresponds to large areas of shared suitable habitat predicted by the species distribution models. Likewise, admixture analysis of the microsatellite data suggests high levels of nuclear gene flow between populations. When combined, these results suggest that the mtDNA clades are likely the result of historical divergence followed by contemporary gene flow. Moreover, this study highlights the importance of incorporating multiple lines of evidence for populations suspected of being cryptic species.

A Dissolved Oxygen Microprobe for Measuring Gut Anaerobic Fermentation in Developing Vertebrates. KATHERINE BACHMAN, KELSEY CARNAHAN, and RICHARD D. DURTSCHE, Department of Biological Sciences, Northern Kentucky University, Highland Heights, KY 41099.

Food assimilation in organisms is important in the extraction of energy. Previous research by both our lab and others suggest anurans are herbivorous and detritivorous feeders. The extent to which cellulose breakdown and fermentation occurs in the GI tract is unknown. Studies done on pH in the GI tract of *Lithobates clamitans* has shown two major drops in pH, in the stomach and hindgut. The use of a dissolved oxygen microelectrode to measure oxygen levels in the gut would give us an idea of possible locations of cellulose fermentation and the possible existence of a colon. Over the past year, our lab has been working on building a dissolved oxygen microelectrode probe to measure these levels. Assembly of these probes is comprised of four main components: the cathode, anode, electrolyte solution and outer casing. The cathode is a glass fused solid-state capillary tube, with a gold tip. The anode is silver wire coated in chloride ions. The outer casing is a glass pipette with a tip diameter of 10 micrometers and gas permeable membrane. The electrolyte is a mixed potassium chloride solution maintained at a high pH. These probes were calibrated with a high degree of accuracy and precision with rapid response times using standard concentrations of dissolved oxygen in water. Regression analyses against standard solutions have R^2 values in the range of 95%. Gastrointestinal tract samples were obtained from *Lithobates clamitans* and readings taken from 15 positions along the gut.

Amphibian Population Dynamics of a Rejuvenating Brown Field. JAMES 'MITCH' MERCER, and RICHARD D. DURTSCHE, Department of Biological Sciences, Northern Kentucky University, Highland Heights, KY 41099.

The Lafarge Gypsum Plant, located in Silver Grove, KY, includes both secondary growth forest consisting of varied hardwood species and open wetland habitats. Just south of the Ohio River, grassland between the secondary forest and the riverbank supports vernal ponds, inundated with water during the spring. Approximately 5 cm of top soil exists due to the land that once served as a railroad yard, the underlying soil being permeated with rock. Determined a brown field during this stage development, chemicals due to industrial waste may remain on site despite rejuvenation efforts. Amphibians are especially susceptible to these environmental conditions as their moist skin facilitates a plethora of life processes. The study was conducted to analyze the ability of the site to sustain wildlife post-rejuvenation efforts. A comparative non-impacted wetland site (St. Anne Wetlands) approximately 2 km away was added to compare species thriving at either location. Various field techniques led to the capture of several species approximately every other day for a span of two years (during months of activity) to understand the dynamics of populations at either site. Species were marked to track migration between sites. Field sound recorders were implemented to track potentially unobserved species. Results suggest that the Lafarge site is able to sustain some reptile and amphibian species, however, several species, in particular salamanders, thrive at the St. Anne wetlands but have not settled at Lafarge.

JUNIOR ACADEMY OF SCIENCE ENGINEERING

Lubrication Efficiency of Oil Weights in Engines. GABRIEL L. M. WEBB-YEATES, Bowling Green High School, Bowling Green, KY 42101.

Engine oils have different weights such as 5W-30 and 10W-30. Each oil weight has a different viscosity and lubrication ability. High weight oil is more viscous. Clean oil should be less viscous than used oil. My hypothesis is that lower weight oils will lubricate better at cold

temperatures. Clean oil should lubricate better than dirty oil. The relative viscosity of different weights of oil from the same manufacturer increased for heavier weights. For the same weight of oil from different manufacturers, the relative viscosity is similar. Used engine oil was more viscous. Lubrication ability was measured by putting a fixed volume of oil between two metal plates placed on a self-manufactured adjustable inclined plane. The height of the inclined plane when the top metal plate starts to slide or slip over the bottom plate was used to measure relative lubrication. Engine oil of the same weight from different companies was measured. Different weights of oils from the same company were measured. Both clean and used oil of the same weight were measured to see if lubrication changes as the oil becomes dirty and used. The inclined plane worked well with good consistency in the slip point height. For the same manufacturer, the lightweight 5W-30 oil lubricated better at cold temperatures than heavy weight oils. Clean oil lubricated better than dirty oil. Surprisingly, high mileage engine oil, which will stick to metal parts better, had a higher slip point than other oils of the same weight.

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